Method for HLA typing

The present invention relates to a method for HLA typing by the unambiguous determination of short DNA sequence elements (2-6 bases) at a given position simultaneously on both parental alleles at a selected number of positions in HLA genes, comprised of the steps for each position of a) hybridising a combination of oligonucleotides (primers) complementary to all known sequence variants to a DNA strand upstream of a given position; b) carrying out a primer extension reaction with at least one of the four dNTP substrates substituted by a terminating analog; c) analysing the products by mass spectrometry, with the resulting masses allowing unambiguous identification of the used primers and the added bases. This method is particularly well suited for DNA-based HLA typing and in combination with a suitable selection of sites tested, it is superior in ease of operation to conventional HLA typing methods.

The most important of the genome projects, the complete sequence of the human genome, is finished. This project reveals the complete sequence of the 3 billion bases and the relative positions of all estimated 30.000 genes in this genome. Having this sequence opens unlimited possibilities for the elucidation of gene function and interaction of different genes. In recent years a systematic effort (SNP consortium) has been underway to identify single nucleotide polymorphisms (SNPs) throughout the human genome and so far several million of these differences between different human beings have been identified (dbSNP contained 5.5 million SNPs in October 2003).

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI) has revolutionized the mass spectrometric analysis of biomolecules (Karas, M. & Hillenkamp, F. *Anal. Chem.* **60**, 2299-2301 (1988)). The field of DNA analysis by mass spectrometry was recently extensively reviewed by Tost and Gut (Mass Spectrometry Reviews, **21**, 388-418 (2002)) and Sauer and Gut (Journal of Chromatography B, **782**, 73-87, (2002)). MALDI has been applied to the analysis of DNA in variations that range from the analysis of PCR products to approaches using allele-specific termination to single nucleotide primer extension reactions and sequencing (Liu, Y.-H., *et al. Rapid Commun. Mass Spectrom.* **9**, 735-743 (1995);

Ch'ang, L.-Y., *et al. Rapid Commun. Mass Spectrom.* **9**, 772-774 (1995); Little, D.P., *et al. J. Mol. Med.* **75**, 745-750 (1997); Haff, L. & Smirnov, I.P. *Genome Res.* **7**, 378-388 (1997), Fei, Z., Ono, T. & Smith, L.M. *Nucleic Acids Res.* **26**, 2827-2828 (1998); Ross, P., Hall, L., Smirnov, I. & Haff, L. *Nature Biotech.* **16**, 1347-1351 (1998); Ross, P.L., Lee, K. & Belgrader, P. *Anal. Chem.* **69**, 4197-4202 (1997); Griffin, T.J., Tang, W. & Smith, L.M. *Nature Biotech.* **15**, 1368-1372 (1997); Köster, H., Higgins, G.S & Little, D.P. US Patent 6,043,031). These methods are used to genotype previously identified mutations, SNPs, or insertion/deletions (indels). Spin column purification and/or magnetic bead technology, reversed-phase purification, or ion-exchange resins are frequently applied prior to mass spectrometric analysis.

The GOOD assay (IG Gut et S. Beck: US 6,268,812 ; IG Gut et al: US 6,503,710) is a method for SNP genotyping that uses MALDI mass spectrometry for detection (Sauer et al. 28, e13 and e100 (2000)). Allele-distinction is based on primer extension. In order to make products more amenable to MALDI analysis a substantial part of the primer is removed prior to mass spectrometric analysis. A further element that is included is charge tagging. This means that the final product is conditioned such that it carries either a single positive or a single negative charge. Generally this is achieved by alkylation of a phosphorothioate backbone and in some instances including a quaternary ammonium group to the penultimate base of the primer. The attachment of the quaternary ammonium group gives options for the design of multiplexes - individual SNPs can be moved up or down in the mass spectrum to achieve optimal resolution and separation.

The major histocompatibility complex (MHC) of humans is a cluster of genes on chromosome 6p21. It is of greatest importance as many diseases show association with genes in this region of the genome. All human leukocyte antigen (HLA) coding genes are found in the MHC. The HLA genes are highly variable and implicated in tissue transplantation, immunity and autoimmune disease such as diabetes, psoriasis, lupus, Crohn's disease, colitis, arthritis, and others. The HLA class I genes are HLA-A, HLA-B, HLA-C, ... The HLA class II genes are HLA-DR, HLA-DQ, HLA-DP,....

HLA typing methods differ dramatically in their approaches. Serological tests can be carried out but have only limited resolution. In the last 15 years the DNA sequence of the MHC has been extensively studied and high resolution typing now makes use of a wealth of DNA sequence information. Methods for DNA based HLA typing range from SSA (sequence specific amplification) where combinations of primers that are specific for different alleles are used to carry out PCR (US 5,545,526). Primers are combined in a way that the sizing of the PCR products allows unambiguous assignment of present base combinations. Multiple combinations are used to identify HLA types. The procedure works its way through a tree of combinations starting with a grouping into rough classes from where on further tests are carried out with specific reagents to subdivide in a class. This method is also known as SSP (sequence specific primers). An alternative method is termed SSOP (sequence specific oligonucleotide probes; US 6,503,707). Here a locus specific PCR is carried out followed by hybridisation with sequence specific oligonucleotide probes. As sequencing technology (and in particular the software for sequence calling) has dramatically improved over the last decade it now is also possible to gain a good degree of identification of HLA types by sequencing (WO 98/35059). Effectively a locus-specific PCR product is sequenced. Problems that arise here are that heterozygous individuals occasionally give rise to ambiguous haplotype calls that can not be resolved (Robinson, J.; Waller, M.J.; Marsh, St.G.E.: "Exon Identities and Ambiguous Typing Combinations"; IMGT/HLA Database; October 2003). The inclusion of allele-specific PCR helps achieve certainty. Resolution requires multiple products per locus to be generated and sequenced. However, as sequencing results can be very convoluted the interpretation in absence of allele-specific PCR can be cumbersome. All together the sequence-based typing requires many iterations in application. Reference strand mediated conformation analysis (RSCA) is a method used to study samples that potentially have a previously unknown sequence in their HLA (Correl et al., Tissue Antigens 56, 82-86, 2000). For a recent review for the reasoning of HLA typing as well as methodological advances see Petersdorf et al. (Tissue Antigens, 61, 1-11, 2003).

The inventors have thus set themselves the task of providing an easy method for the simultaneous capture of all parental mini-haplotypes in highly polymorphic regions of genomes. The procedure has to be executable on a cost-effective genotyping platform. The method should be particularly applicable for HLA typing. It is an aim to resolve frequent and rare HLA alleles as well as possible.

The object of the present invention is a method for HLA typing by the unambiguous determination of short DNA sequence elements (2-6 bases) simultaneously on both parental alleles at a selected number of positions in HLA genes, comprised of the steps for each position of a) hybridising a combination of oligonucleotides (primer pool) complementary to all known sequence variants to a DNA strand upstream of a given position; b) carrying out a primer extension reaction with at least one of the four dNTP substrates substituted by a terminating analog; c) analysing the products by mass spectrometry, with the resulting masses allowing unambiguous identification of the used primers and the added bases.

In the present invention:

- "HLA" means the human leukocyte antigen locus on chromosome 6p21, consisting of HLA genes (HLA-A, HLA-B, HLA-C, HLA-DRB1,...) that are used to determine the degree of matching, for example, between a recipient and a donor of a tissue graft.
- "HLA typing" means the identification of a known HLA allele of a given locus (HLA-A, HLA-B, HLA-C, HLA-DRB1,...).
- "HLA allele" means a nucleotide sequence within a locus on one of the two parental chromosomes.
- "HLA-A" means the DNA sequence of exons 2 and 3 of the HLA-A gene.
- "HLA-B" means the DNA sequence of exons 2 and 3 of the HLA-B gene.
- "HLA-DRB1" means the DNA sequence of exon 2 of the HLA-DRB1 gene.
- "Polymorphism" means individual positions in a DNA sequence that exist in different variants.
- "Haplotype" means the DNA sequence of one of the two alleles in a give region of the genome.

- "Mini-haplotype" means 2-6 contiguous bases on one parental allele.
- "Primer pools" or "pools of primers" means sets of primers that are used in one primer extension reaction. For each known HLA allele at least one primer is in the pool that is completely complementary in sequence. This assures perfect annealing. Mismatches that are more than 4 bases from the 3'end of the primer do not affect the results of the GOOD assay, as all of those bases are removed by 5'phosphodiesterase after the primer extension reaction. Primers of the pool containing mismatches in the last few bases are not extended by the DNA polymerase and thus not observable.
- "MALDI mass spectrometer" means a mass spectrometer that uses matrix-assisted laser desorption/ionization for the volatilisation of a sample and time-of-flight analysis for mass separation.
- "Subgroup" means alleles, which are identical after the mini-haplotyping of the first set of selected positions. For the high resolution typing we resolve subgroups generated with 10 mini-haplotyping reactions. The criteria for resolving subgroups are: a) they still contain alleles with different two-digit types, b) subgroups with more than four alleles, and c) subgroups with frequent alleles (see list below).

Here we show a methodology for the determination of sequence motifs of 2-6 bases in very polymorphic regions of genomes. In principle this methods equates to the determination of mini-haplotypes of 2-6 bases. The individual parental mini-haplotypes can be determined in one reaction without ambiguities. This methodology is applied to a chosen set of positions for HLA typing of HLA-A, HLA-B, and HLA-DRB1. The sets disclosed here have different purposes. First sets of 19, 19, and 10 positions are suggested to distinguish a maximum of HLA alleles in HLA-A, HLA-B, and HLA-DRB1, respectively, with respect to differentiating alleles that are frequent in the general population from ones that are rare. The frequent alleles that were screened for are A*0101, A*0201, A*0301, A*2301, A*2402, A*2902, A*3001 and A*3002 for HLA-A, B*0702, B*0801, B*1302, B*1501, B*1801, B*3501, B*3503, B*4001, B*4402, B*4403, B*5101 and B*5701 for HLA-B, and DRB1*0101, DRB1*0301, DRB1*0401, DRB1*0701,

DRB1*1101, DRB1*1104, DRB1*1302 and DRB1*1501 for HLA-DRB1. This set of markers provides unambiguous identification of frequent HLA alleles with 93.4 - 100 % certainty in HLA-A, 97.6 - 100 % in HLA-B, and 97.2 - 100 % in HLA-DRB1.

A second set of 10 positions each in HLA-A, HLA-B, and HLA-DRB1, respectively are described that provide a maximum number of subgroups, that can then be further resolved by the addition of a set of subgroup specific positions. Again the ten positions in each locus were chosen on the basis of providing best distinction between the frequent HLA alleles listed above from the rest of the HLA alleles (rare). This resulted in groups containing 2-30 HLA alleles depending on the locus. Within each group a number of positions can be tested to provide resolution between the HLA alleles within the group. The number of positions that have to be additionally analysed range from 1-25 in order to achieve 4-digit resolution. With this technology HLA typing can be carried out at a substantially reduced cost with a proven high-throughput detection platform (MALDI mass spectrometry).

In a preferred embodiment of the method of the invention, the DNA strand of step a) is produced by a DNA replication procedure such as PCR or rolling circle replication.

A set of locus-specific PCR reactions for the selective amplification of each locus is described by the International Histocompatibility Working Group, Technical Manuals (www.ihwg.org/tmanual/Tmcontents.htm).

In a very preferred embodiment of the method of the invention, a combination of primers (pools of primers) contains slightly varying sequences so that all known sequences of the HLA alleles are accommodated by a perfectly matching primer. The pool of primers guarantees that at least one primer is perfectly matched. The hybridised oligonucleotides of the primer pool are extended onto a polymorphic position. A requirement is that the added base together with the base composition of the primer gives a unique mass. The detection of this mass in the mass spectrometric profile indicates the presence of a sequence containing both the complementary sequence of the primer and the added base. In order to make all primers of a primer pool distinguishable by mass it is possible to add different mass

shifting agents to the primers. The easiest way to accomplish this is by using charge/mass tagging technology such as is used in the GOOD assay. The penultimate base from the 3'end of the primer is amino-modified and used to add tags via NHS-ester chemistry. The pools of primers of course contain primers that sometimes differ by as little as one base. Sequences identical in base content can still be distinguished by the suitable selection of mass tags. Also, we have found that a primer carrying a mismatch in the last eight bases from the 3'end even if it anneals is not extended by the polymerase and thus screened out. This might be due to insufficient hybridisation or a resistance of the DNA polymerase to attach or extend when a mismatch is present. We thus make use of two effects for our mini-haplotyping: 1) allele-specific hybridisation and 2) allele-specific primer extension. Mismatches that are further than four bases away from the 3'end of the extension primer do not result in increased complexity of the mass spectra as they are removed in the 5'phosphodiesterase digestion step of the GOOD assay.

In a preferred embodiment of the method of the invention, mass shifting tags are added to the individual primers sequences of a primer pool to make them uniquely distinguishable once the terminating base is added.

In another preferred embodiment of the method of the invention, termination products for know alleles are generated by extending the perfectly hybridised primer with a combination of dNTPs and ddNTPs or analogues thereof with a DNA polymerase to generate specific termination products to make them uniquely distinguishable by their mass.

In a preferred embodiment of the method of the invention, the GOOD assay is used. It typically applies single base primer extension, thus only the four terminating bases (ddNTPs) or synthetic analogues with the same qualities in terms of DNA polymerase tolerance are used for primer extension. α-S-ddNTPs are very suitable analogues.

In a preferred embodiment of the method of the invention, mass spectrometry, in particular MALDI or ESI mass spectrometry is used for analysis of the masses of products.

For HLA typing a set of said mini-haplotyping assays has to be carried out to achieve sufficient information content.

For HLA typing of HLA-A the preferred set of assays are those of positions 98, 414, 539, 282, 571, 368, 256, 292, 238, 270, 453, 527, 502, 81, 268, 559, 92, 123 and 396 (according to the numbering of the HLA-A gene starting at cDNA sequence position 1 of exon 1; see Figure 1). This results in medium resolution HLA typing. The input criteria for the selection are the frequency of HLA alleles. Some HLA types are identified unambiguously.

For HLA typing of HLA-B accordingly the following positions are preferably analysed by mini-haplotyping assays to achieve medium resolution: 539, 419, 559, 412, 272, 362, 302, 363, 206, 369, 259, 97, 583, 292, 222, 527, 418, 435 and 571 (according to the numbering of the HLA-B gene starting at cDNA sequence position 1 of exon 1; see Figure 2).

For HLA typing of HLA-DRB1 accordingly the following positions are preferably analysed by mini-haplotyping to achieve medium resolution: 125, 196, 197, 227, 261, 286, 299, 308, 341 and 345 (according to the numbering of the HLA-DRB1 gene starting at cDNA sequence position 1 of exon 1; see Figure 3).

In a preferred embodiment for high resolution HLA typing of HLA-A positions 98, 414, 539, 282, 571, 368, 256, 292, 238 and 270 (according to the numbering of the HLA-A gene starting at cDNA sequence position 1 of exon 1; see Figure 4) are used for mini-haplotyping to generate sub-groups (HLA-A_A, HLA-A_B, HLA-A_C, HLA-A_D, HLA-A_E, HLA-A_F, HLA-A_G, HLA-A_H, HLA-A_I, HLA-A_J, HLA-A_K, HLA-A_L, HLA-A_M, HLA-A_N, and HLA-A_O; see Table I).

Positions 224, 268, 376, 502, 561 and 616 are preferably analysed to resolve subgroup HLA-A_A (sequences identical over exons 2 and 3 for alleles A*29010101 and A*29010102); positions 126 and 526 to resolve subgroup HLA-A_B; positions 81, 90, 92, 212, 214, 257, 265, 299, 302, 404, 420, 427, 453, 485, 489 and 502 to resolve subgroup HLA-A_C (sequences identical over exons 2 and 3 for alleles A*24020101, A*24020102L, A*240203, A*2409N and A*2411N); positions 160, 200, 362 and 524 to resolve subgroup HLA-A_D; positions 180, 299, 301, 302, 346, 418, 453, 517, 524, 526, 527, 557, 559 and 560 to resolve subgroup HLA-A_E; positions 299, 301, 302, 341 and 583 to resolve subgroup HLA-A_F; positions 127, 341, 399, 480, 502, 503, 524, 526, 527, 553, 559, 560 and 565 to resolve subgroup HLA-A_G; positions 228, 233, 463, 519, 530 and 583 to resolve

subgroup HLA-A_H; positions 102, 275, 317, 362, 418, 419, 497, 524, 555, 595 and 618 to resolve subgroup HLA-A_I (sequences identical over exons 2 and 3 for alleles A*680102 and A*6811N); positions 92, 331, 453, 524, 559, 560 and 564 to resolve subgroup HLA-A_J; positions 78, 81, 123, 125, 142, 144, 194, 268, 294, 324, 355, 362, 396, 403, 419, 453, 456, 477, 493, 517, 524, 526, 527, 559 and 560 to resolve subgroup HLA-A_K (sequences identical over exons 2 and 3 for alleles A*02010101, A*02010102, A*020108, A*0209, A*0243N and A*0266); positions 113, 299, 301, 302, 308, 311, 523, 524 to resolve subgroup HLA-A_L; positions 171, 363, 498 and 559 to resolve subgroup HLA-A_M; positions 376, 426, 527, 555, 557 and 595 to resolve subgroup HLA-A_N; position 299 to resolve subgroup HLA-A_O.

TABLE I

Subgroups of HLA-A	Alleles of Subgroups	Positions to resolve Subgroups
HLA-A_A	A*29010101, A*29010102, A*290201, A*290202, A*2904, A*2906, A*2908N, A*2909	224, 268, 376, 502, 561, 616
HLA-A_B	A*3002, A*3009, A*3012	126, 526
HLA-A_C	A*24020101, A*24020102L, A*240202, A*240203, A*240204, A*2404, A*2405, A*2408, A*2409N, A*2411N, A*2420, A*2421, A*2425, A*2426, A*2427, A*2429, A*2432, A*2435, A*2436N, A*2437, A*2438, A*2439	81, 90, 92, 212, 214, 257, 265, 299, 302, 404, 420, 427, 453, 485, 485, 489, 502
HLA-A_D	A*0206, A*0214, A*0221, A*0251, A*0257	160, 200, 362, 524
HLA-A_E	A*250101, A*250102, A*2601, A*2604, A*2605, A*2609, A*2610, A*2611N, A*2612, A*2614, A*2615, A*2617, A*2618, A*6603	180, 299, 301, 302, 346, 418, 453, 517, 524, 526, 527, 557, 559, 560
HLA-A_F	A*2502, A*2613, A*6601, A*6602, A*6604	299, 301, 302, 341, 583
HLA-A_G	A*110101, A*110102, A*1102, A*1103, A*1104, A*1105, A*1107, A*1109, A*1112, A*1113, A*1114, A*1115	127, 341, 399, 480, 502, 503, 524, 526, 527, 553, 559, 560, 565
HLA-A_H	A*3301, A*330301, A*330302, A*3304, A*3305, A*3306, A*3307	228, 233, 463, 519, 530, 583
HLA-A_I	A*680101, A*680102, A*680103, A*6807, A*6811N, A*6812, A*6816, A*6817, A*6819, A*6821, A*6822, A*6823, A*6824	102, 275, 317, 362, 418, 419, 497, 524, 555, 595, 618
HLA-A_J	A*2301, A*2303, A*2305, A*2306, A*2307N, A*2308N, A*2310, A*2413	92, 331, 453, 524, 556, 560, 564
HLA-A_K	A*02010101, A*02010102, A*020102, A*020103, A*020104, A*020105, A*020106, A*020107, A*020108, A*020109, A*0204, A*0209, A*0216, A*0224, A*0225, A*0226, A*0229, A*0230, A*0231, A*0232N, 0A*0240, A*0242, A*0243N, A*0258, A*0259, A*0260, A*0264, A*0266, A*0267, A*0253N	78, 81, 123, 125, 142, 144, 194, 268, 294, 324, 355, 362, 396, 403, 419, 453, 419, 453, 456, 477, 493, 517, 524, 526, 527, 559, 560
HLA-A_L	A*3201, A*3203, A*3206, A*7401, A*7402, A*7403, A*7408, A*7409	113, 299, 301, 302, 308, 311, 523, 524
HLA-A_M	A*010101, A*010102, A*0103, A*0104N, A*0108, A*0109	171, 363, 498, 559
HLA-A_N	A*03010101, A*03010102, A*0303N, A*0304, A*0305, A*0306, A*0307, A*0311N	376, 426, 527, 555, 557, 595
HLA-A_O	A*2504, A*2608	299

In a preferred embodiment for high resolution, HLA typing of HLA-B positions 539, 419, 559, 412, 272, 362, 302, 363, 206 and 369 (according to the numbering of the HLA-B gene starting at cDNA sequence position 1 of exon 1; see Figure 5) are used for mini-haplotyping to generate sub-groups (HLA-B_A, HLA-B_B, HLA-B_C, HLA-B_D, HLA-B_E, HLA-B_F, HLA-B_G, HLA-B_H, HLA-B_I, HLA-B_J, HLA-B_K, HLA-B_L, HLA-B_M, HLA-B_N, HLA-B_O, HLA-B_P, HLA-B_Q, HLA-B_R, HLA-B_S, HLA-B_T, HLA-B_U, HLA-B_V, HLA-B_W, HLA-B_X, HLA-B_Y, HLA-B_Z, HLA-B_AA, HLA-B_AB and HLA-B_AC ; see Table II).

Positions 259, 341 and 473 are preferably analyzed to resolve subgroup HLA-B_A (sequences identical over exons 2 and 3 for alleles B*0801 and B*0819N); positions 106, 144, 222, 259, 273, 311, 313, 418, 445, 493, 528 and 540 to resolve subgroup HLA-B_B (sequences identical over exons 2 and 3 for alleles B*44020101, B*44020102, B*4419N and B*4427); positions 319, 416, 545 and 572 to resolve subgroup HLA-B_C; positions 106, 131, 165, 215, 243, 277, 292, 322, 481, 582, 603 and 616 to resolve subgroup HLA-B_D; positions 106, 146, 165, 181, 238, 259, 263, 292, 328.1/329(insert for B*1579N), 379, 435, 453, 463, 485, 526, 571, 572 and 583 to resolve subgroup HLA-B_E (sequences identical over exons 2 and 3 for alleles B*15010101 and B*15010102); positions 142, 171, 255, 257, 395, 430, 544, 566 and 572 to resolve subgroup HLA-B_F; positions 117, 247, 248, 277, 345, 418, 489 and 527 to resolve subgroup HLA-B_G (sequences identical over exons 2 and 3 for alleles B*270502, B*270504 and B*2713); positions 134, 141, 200, 213, 259, 304 and 527 to resolve subgroup HLA-B_H; positions 83, 141, 211, 222, 242, 322, 404, 414, 435, 463, 502, 527, 544, 571, 572 and 583 to resolve subgroup HLA-B_I (sequences identical over exons 2 and for alleles B*510101, B*510105, B*5111N, B*5130 and B*5132); positions 103, 142, 222, 243, 259, 292, 477, 486 and 499 to resolve subgroup HLA-B_J (sequences identical over exons 2 and 3 for alleles B*400101 and B*400102); positions 103, 259, 292, 295, 527 and 583 to resolve subgroup HLA-B_K (sequences identical over exons 2 and 3 for alleles B*180101 and B*1817N); positions 320 and 500 to resolve subgroup HLA-B_L; positions 311, 527 and 583 to resolve subgroup HLA-B_M; positions 119, 292, 259, 319, 425, 527, 546 and 583 to resolve subgroup HLA-B_N (sequences identical over exons 2 and 3 for alleles B*350101, B*3540N and B*3542); positions 97, 142, 245 and 527 to resolve subgroup HLA-B_O; positions 97 and 175 to resolve subgroup HLA-B_P; positions

TABLE II

Subgroups of HLA-B	*Alleles of the subgroup*	*Positions to resolve Subgroups*
HLA-B_A	B*0801, B*0808N, B*0810, B*0818, B*0819N	259, 341, 473
HLA-B_B	B*44020101, B*44020102S, B*440202, B*440203, B*4405, B*4411, B*4412, B*4419N, B*4422, B*4423N, B*4424, B*4425, B*4427, B*4433, B*4434, B*4435	106, 144, 222, 259, 273 311, 313, 418 445, 493, 528, 540
HLA-B_C	B*4415, B*4501, B*4503, B*4504, B*4505	319, 416, 545, 572
HLA-B_D	B*070201, B*070202, B*070203, B*070204, B*0703, B*0716, B*0721, B*0722, B*0723, B*0729, B*0730, B*0733, B*0735	106, 131, 165, 215, 243, 277, 292, 322, 481, 582, 603 , 616
HLA-B_E	B*15010101, B*15010102, B*150102, B*150103, B*150104, B*1512, B*1514, B*1515, B*1519, B*1528, B*1533, B*1534, B*1538, B*1560, B*1570, B*1571, B*1575, B*1578, B*1579N, B*1581, B*1582	106, 146, 165, 181, 238, 259, 263, 292, 328.1/329, 379, 435, 453, 463, 485, 526, 571, 572 , 583
HLA-B_F	B*440301, B*4413, B*4426, B*4429, B*4430, B*4432, B*4436, B*4437, B*4438, B*4439	142, 171, 255, 257, 395, 430, 544, 566 , 572
HLA-B_G	B*2703, B*270502, B*270503, B*270504, B*270505, B*270506, B*2709, B*2710, B*2713, B*2716, B*2717	117, 247, 248, 277, 345, 418, 489 , 527
HLA-B_H	B*5107, B*520101, B*520102, B*520103, B*520104, B*5203, B*5204, B*5205	134, 141, 200, 213, 259, 304 , 527
HLA-B_I	B*510101, B*510102, B*510103, B*510104, B*510105, B*510201, B*510202, B*5103, B*5109, B*5111N, B*5112, B*5114, B*5118, B*5119, B*5123, B*5124, B*5126, B*5127N, B*5128, B*5130, B*5132, B*5133	83, 141, 211, 222, 242, 322, 404, 414, 435, 463, 502, 527, 544, 571, 572 , 583
HLA-B_J	B*400101, B*400102, B*400103, B*4010, B*4011, B*401401, B*401402, B*401403, B*4022N, B*4025, B*4043	103, 142, 222, 243, 259, 292, 477, 486 , 499
HLA-B_K	B*180101, B*180102, B*1803, B*1804, B*1805, B*1811, B*1812, B*1815, B*1817N	103, 259, 292, 295, 527 , 583
HLA-B_L	B*570101, B*5706, B*5708	320, 500
HLA-B_M	B*3527, B*5301, B*5302, B*5306, B*5308	311, 527 , 583
HLA-B_N	B*350101, B*350102, B*3507, B*3510, B*3511, B*3521, B*3524, B*3529, B*3540N, B*3541, B*3542, B*5305	119, 292, 259, 319, 425, 527, 546 , 583
HLA-B_O	B*5501, B*5502, B*5505, B*5510, B*5516	97, 142, 245, 527
HLA-B_P	B*5401, B*5402, B*5507	97 , 175

HLA-B_Q	B*3910, B*670101, B*670102	246, 277
HLA-B_R	B*3803, B*390201, B*390202, B*3913, B*3923	246, 292, 311 , 503
HLA-B_S	B*3801, B*380201, B*380202, B*3804, B*3805, B*3809	103, 261, 309, 311 , 474
HLA-B_T	B*390101, B*390103, B*390104, B*3904, B*3905, B*3912, B*3922, B*3925N, B*3926	97, 103, 106, 243, 259, 292, 404 , 524
HLA-B_U	B*3503, B*3513, B*3536	259 , 320
HLA-B_V	B*0734, B*5504	106
HLA-B_W	B*4047, B* 4431	97
HLA-B_X	B*4002, B*4027, B*4029, B*4035, B*4040, B*4045	97, 106, 257, 418, 463
HLA-B_Y	B*400104, B*4004	106
HLA-B_Z	B*4012, B*4046, B*4803	106, 144
HLA-B_AA	B*2703, B*270502, B*270503, B*270504, B*270505, B*270506, B*2709, B*2710, B*2713, B*2716, B*2717	117, 247, 248, 283, 345, 418, 489, 527
HLA-B_AB	B*1562, B*4802	106
HLA-B_AC	B*1302, B*1308	548

246 and 277 to resolve subgroup HLA-B_Q; positions 246, 292, 311 and 503 to resolve subgroup HLA-B_R; positions 103, 261, 309, 311 and 474 to resolve subgroup HLA-B_S; positions 97, 103, 106, 243, 259, 292, 404 and 524 to resolve subgroup HLA-B_T (sequences identical over exons 2 and 3 for alleles B*390101 and B*390103); positions 259 and 320 to resolve subgroup HLA-B_U; position 106 to resolve HLA-B_V; positions 97 to resolve HLA-B_W; positions 97, 106, 257, 418 and 463 to resolve HLA-B_X; position 106 to resolve HLA-B_Y; positions 106 and 144 to resolve HLA-B_Z; positions 117, 247, 248, 283, 345, 418, 489, and 527 to resolve HLA-B_AA; positions 106 to resolve HLA-B_AB; positions 548 to resolve HLA-B_AC.

In a preferred embodiment, the method for HLA typing resolves groups A-P of HLA-DRB1.

For high resolution, HLA typing of HLA-DRB1 positions are: 125, 196, 197, 227, 261, 286, 299, 308, 341 and 345 (according to the numbering of the HLA-DRB1 gene starting at DNA sequence position 1 of exon 1; see Figure 6) are used for mini-haplotyping to generate sub-groups (HLA-DRB1_A, HLA-DRB1_B, HLA-DRB1_C, HLA-DRB1_D, HLA-DRB1_E, HLA-DRB1_F, HLA-DRB1_G, HLA-DRB1_H, HLA-DRB1_I, HLA-DRB1_J, HLA-DRB1_K, HLA-DRB1_L, HLA-DRB1_M, HLA-DRB1_N, HLA-DRB1_O, HLA-DRB1_P; see Table III).

In a very preferred embodiment, positions 123, 174, 250, 278 and 317 are analysed to resolve subgroup HLA-DRB1_A; positions 192, 203, 256 and 259 to resolve subgroup HLA-DRB1_B; 256, 260, 317 and 351 to resolve subgroup HLA-DRB1_C; positions 155, 204, 233, 239, 256, 304, 357 and 366 to resolve subgroup HLA-DRB1_D; positions 122, 171, 257 and 317 to resolve subgroup HLA-DRB1_E; positions 164, 167, 171, 230, 235, 306, 317, 321 and 337 to resolve subgroup HLA-DRB1_F; positions 164, 257, 266 and 303 to resolve subgroup HLA-DRB1_G; positions 164, 181, 188, 220, 229, 256, 266, 317 and 318 to resolve subgroup HLA-DRB1_H; position 257 to resolve subgroup HLA-DRB1_I; positions 181, 239 and 357 to resolve subgroup HLA-DRB1_J; positions 122, 144, 239, 303, 317, 318 and 321 to resolve subgroup HLA-DRB1_K (sequences identical over exons 2 and 3 for alleles DRB1*110101 and DRB1*110102); positions 118, 161, 257, 260, 318 and 321 to resolve subgroup HLA-DRB1_L; positions 165, 257, 293 and 303 to resolve subgroup HLA-DRB1_M (sequences identical over exons 2 and 3 for alleles DRB1*120101 and DRB1*1206); positions 177, 240, 256, 257 and 357 to resolve subgroup HLA-DRB1_N; positions 150 175, 230, 236 and 321 to resolve subgroup HLA-DRB1_O (sequences identical over exons 2 and 3 for alleles DRB1*150101 and DRB1*1513); positions 115, 220 and 317 to resolve subgroup HLA-DRB1_P.

Another object of the invention is a kit to carry out the procedure. It consists of pooled combinations of primers. The primers that are used in the pools for HLA-A, HLA-B, and HLA-DRB1 and the masses of the genotyping products are listed in Tables IV, V, and VI respectively. CT refers to the mass shifting mass tag that is attached to that primer of the pool.

Another object of the invention is the use of the method of the invention for screening of tissue donors.

In a preferred embodiment, the use is for bone marrow donors in registries for screening of frequent and rare HLA types.

Still another object of the invention is the use of the primers represented in Table IV, V and VI to carry out HLA typing.

TABLE III

Subgroups of HLA-DRB1	Alleles of Subgroups	Positions to resolve Subgroups
HLA-DRB1_A	DRB1*070101, DRB1*070102, DRB1*0703, DRB1*0704, DRB1*0705, DRB1*0707	123, 174, 250, 317
HLA-DRB1_B	DRB1*040101, DRB1*040102, DRB1*0409, DRB1*0426, DRB1*0433	192, 203, 256, 259
HLA-DRB1_C	DRB1*0404, DRB1*0410, DRB1*0423, DRB1*0440, DRB1*0444	256, 260, 317, 351.
HLA-DRB1_D	DRB1*040501, DRB1*040502, DRB1*040503, DRB1*040504, DRB1*0408, DRB1*0429, DRB1*0430, DRB1*0445, DRB1*0448	155, 204, 233, 239, 256, 304, 357, 366
HLA-DRB1_E	DRB1*1402, DRB1*1409, DRB1*1413, DRB1*1446, DRB1*1447, DRB1*1448	122, 171, 257, 317
HLA-DRB1_F	DRB1*130101, DRB1*130102, DRB1*130103, DRB1*1315, DRB1* 1327,	164, 167, 171, 230, 235, 306, 317, 321, 337
HLA-DRB1_G	DRB1*130201, DRB1*130202, DRB1*1331, DRB1*1339, DRB1*1341	164, 257, 266, 303
HLA-DRB1_H	DRB1*030101, DRB1*030102, DRB1*0307, DRB1*0312, DRB1*0313, DRB1*0315, DRB1*0316, DRB1*0318, DRB1*0322, DRB1*0323	164, 181, 188, 220, 229, 256, 266, 317, 318
HLA-DRB1_I	DRB1*1137, DRB1*1425	257
HLA-DRB1_J	DRB1*110401, DRB1*110402, DRB1*1143, DRB1*1146	181, 239, 357
HLA-DRB1 _K	DRB1*110101, DRB1*110102, DRB1*110103, DRB1*110104, DRB1*110105, DRB1*112701, DRB1*112702, DRB1*1130, DRB1*1139	122, 144, 239, 303, 317, 318, 321
HLA-DRB1_L	DRB1*1117, DRB1*140101, DRB1*140102, DRB1*1408, DRB1*1426, DRB1*1438, DRB1*1439	118, 161, 257, 260, 318, 321
HLA-DRB1_M	DRB1*120101, DRB1*120102, DRB1*1206, DRB1*1207, DRB1*1208, DRB1*1209	165, 257, 293, 303
HLA-DRB1_N	DRB1*080101, DRB1*080102, DRB1*080201, DRB1*080202, DRB1*080203, DRB1*0807, DRB1*0811	177, 240, 256, 257, 357
HLA-DRB1_O	DRB1*150101, DRB1*150103, DRB1*150105, DRB1*1503, DRB1*1506, DRB1*1509, DRB1*1513	150 175, 230, 236, 321
HLA-DRB1_P	DRB1*010101, DRB1*0105, DRB1*0107, DRB1*0111	115, 220, 317

TABLE IV

No.	Name	Sequence	CT	Primer Masses	A	C	G	T
1	HLAA_811_1f20	TGCTCGCCCCCAGGCTCCspC^spA	0	1098,1	1425,1	1401,3	-	-
2	HLAA_812_1f20	TGCTCGCCCCCAGGCTCTspC^spA	0	1113,1	-	1416,3	1452,4	-
3	HLAA_921_1f20	AGGCTCCCACTCCATGAGspC^spT	0	1129,1	1456,4	-	-	-
4	HLAA_922_1f20	AGGCTCCCAMTCCATGAGspG^spT	0	1169,1	1496,4	-	1512,4	-
5	HLAA_923_1f20	AGGCTCTCASTCCATGAGspG^spT	0	1169,1	1496,4	-	1512,4	-
6	HLAA_981_1f20	CCACTCCATGAGGTATTTspC^spA	0	1113,1	-	1416,3	-	-
7	HLAA_982_1f20	CCACTCCATGAGGTATTTspC^spT	0	1104,1	1431,4	1407,3	-	1422,3
8	HLAA_1231_2r20	GCGATGAAGCGGGGCTCspCspT^spC	0	1510,5	-	-	1853,8	-
9	HLAA_1232_2r20	GCGATGAAGCGGGGCTCspTspC^spC	-28	1380,4	1707,7	-	-	-
10	HLAA_1233_2r20	GCGATGAAGCGGGGCTTspCspC^spC	0	1408,4	-	-	1751,6	-
11	HLAA_1234_2r20	GMGATGAAGCGGGGCTCspCspC^spC	0	1393,4	1720,7	-	1736,7	-
12	HLAA_2381_2r20	CTSGTCCCAATACTCCGspGspA^spC	0	1497,4	-	1800,6	-	-
13	HLAA_2382_2r20	CYCGTCCCAATACTCCGspGspA^spC	0	1497,4	-	1800,6	-	-
14	HLAA_2383_2r20	CTCGTCCCAATACTCCGspGspC^spT	0	1488,4	-	1791,6	-	1806,4
15	HLAA_2384_2r20	CTSGTCCCAATACTCAGspGspC^spC	0	1473,4	-	1776,6	-	-
16	HLAA_2385_2r20	CYGGTCCCAATACTCCGspGspC^spC	0	1473,4	-	1776,6	-	-
17	HLAA_2386_2r20	CMGGTCCCAATACTCCGspGspC^spC	0	1473,4	-	1776,6	-	-
18	HLAA_2387_2r20	CYCGTCCCAATACTCCGspGspC^spC	0	1473,4	-	1776,6	-	-
19	HLAA_2561_1r19	CTTCATATTCCGTGTCTCspC^spT	0	1089,1	-	1392,3	1432,4	-
20	HLAA_2562_1r19	CTTCACWTTCCGTGTCTCspC^spT	0	1089,1	-	1392,3	1432,4	-
21	HLAA_2563_1r19	CTTCACATKCCGTGTCTGspC^spA	0	1138,1	-	-	1481,4	-
22	HLAA_2564_1r19	CTTCACTTTCCGTGTGTTspC^spC	0	1089,1	-	-	1432,1	-
23	HLAA_2565_1r19	CYTCACATTCCGTGTGTTspC^spC	0	1089,1	-	-	1432,1	-
24	HLAA_2566_1r19	CTTCACRTTCCGTGTCTCspC^spC	0	1074,1	-	1377,3	1417,4	-
25	HLAA_2567_1r19	CTTCASTTGCCGTGTCTCspC^spC	0	1074,1	-	1377,3	1417,4	-
26	HLAA_2568_1r19	CTTCAGTTKCCGTGTCTCspC^spC	0	1074,1	-	1377,3	1417,4	-
28	HLAA_2681_1f20	ATTGGGACCGGAACACACspG^spG	0	1154,1	1481,4	1457,3	-	-
29	HLAA_2682_1f20	ATTGGGACCTGCAGACACspG^spG	0	1154,1	1481,4	1457,3	-	-
30	HLAA_2683_1f20	ATTGGGACSAGGAGACACspG^spG	0	1154,1	1481,4	1457,3	-	-
31	HLAA_2684_1f20	ATTGGGACSGGGAGACACspG^spG	0	1154,1	1481,4	1457,3	-	-
32	HLAA_2685_1f20	ATTGGGACSAGGAGACAGspG^spG	0	1194,1	1521,4	-	-	-
33	HLAA_2701_1r19	CTGTGAGTGGGCCTTCspA^spT	0	1113,1	1440,4	-	-	-
34	HLAA_2702_1r19	CTGTGACTGGGCCYTCspA^spC	-14	1084,1	1411,4	-	1427,4	1402,4
35	HLAA_2703_1r19	CTGTGAGTGGSCCTTCspA^spC	-14	1084,1	1411,4	-	1427,4	1402,4
36	HLAA_2821_1f20	ACACGGAATGTGARGGGCspC^spA	0	1098,1	-	1401,3	1441,3	-
37	HLAA_2822_1f20	ACASGGAAAGTGAAGGCCspC^spA	0	1098,1	-	1401,3	1441,3	-
38	HLAA_2823_1f20	ACACGGCAWGTGAAGGCCspC^spA	0	1098,1	-	1401,3	1441,3	-
39	HLAA_2824_1f20	ACACGGAACGTGAAGGCCspC^spA	0	1098,1	-	1401,3	1441,3	-
40	HLAA_2825_1f20	ACACGGAATRTGAAGGCCspC^spA	0	1098,1	-	1401,3	1441,3	-
41	HLAA_2921_2f20	TGAAGGCCCACTCACAGspAspG^spT	-14	1498,4	-	1801,6	-	-
42	HLAA_2922_2f20	TGAAGGCCCACTCACAGspGspC^spT	0	1488,4	-	-	1831,7	-
43	HLAA_2923_2f20	TGAAGGSCCACTCACAGspAspT^spT	0	1589,6	-	-	1932,9	-
44	HLAA_2924_2f20	TGARGGCCCAGTCACAGspAspC^spT	0	1427,4	-	1775,6	1815,7	-
45	HLAA_2925_2f20	TGAAGGCCCASTCACAGspAspC^spT	0	1427,4	-	1775,6	1815,7	-
46	HLAA_3681_1f20	TCACACCATCCAGATAATspG^spC	0	1129,1	1456,4	-	-	-
47	HLAA_3682_1f20	TCACACCATCCAGMTAATspG^spT	0	1144,1	1471;6	1447,1	1487,4	1462,3
48	HLAA_3683_1f20	TCACACCSTCCAGAGGATspG^spT	0	1144,1	1471,6	1447,1	1487,4	1462,3
49	HLAA_3684_1f20	TCACACCVTCCAGATGATspG^spT	0	1144,1	1471,6	1447,1	1487,4	1462,3
50	HLAA_3961_2r20	GCTGGTACCCGCGGAGspGspA^spG	0	1537,4	-	-	1880,7	-

51	HLAA_3962_2r20	GCCGGTACCCGCGGAGspTspA^spA	0	1496,4	-	-	1839,7	-
52	HLAA_3963_2r20	GGTGGTACCCGYGCAGspGspA^spA	0	1496,4	-	-	1839,7	-
53	HLAA_3964_2r20	GGTGGTACCCGCAGAGspGspA^spA	0	1521,5	-	-	1864,8	1839,7
54	HLAA_3965_2r20	GTTCATACCCGCGGAGspGspA^spA	0	1521,5	-	-	1864,8	1839,7
55	HLAA_3966_2r20	GSTGGTACCCGCGGAGspGspA^spA	0	1521,5	-	-	1864,8	1839,7
56	HLAA_3967_2r20	GCCGGTACCCGCGGAGspGspA^spA	0	1521,5	-	-	1864,8	1839,7
57	HLAA_4141_1f20	CGCTTCCTCCGCGGGTATspG^spA	0	1153,1	1480,1	-	-	-
58	HLAA_4142_1f20	CGCTTCCTCTGCGGGTACspC^spA	0	1098,1	-	1401,3	1441,4	-
59	HLAA_4143_1f20	CGCTTCCTGCGCGGGTACspC^spA	0	1098,1	-	1401,3	1441,4	-
60	HLAA_4144_1f20	CGCTTCCTCCACGGGTACspC^spA	0	1098,1	-	1401,3	1441,4	-
61	HLAA_4145_1f20	CGMTTCCTCCGCGGGTACspC^spA	0	1098,1	-	1401,3	1441,4	-
62	HLAA_4146_1f20	CGCCTCCTCCGCGGGTACspC^spA	0	1098,1	-	1401,3	1441,4	-
63	HLAA_4147_1f20	CACTTCCTCCGCGGGTACspC^spG	0	1114,1	-	-	1457,4	-
64	HLAA_4148_1f20	CGCTTMCTCCGCGGGTACspC^spG	0	1114,1	-	-	1457,4	-
65	HLAA_4531_1r20	GTCCAAGAGCGCAGGTCTspT^spC	0	1206,2	-	-	-	1524,4
66	HLAA_4532_1r20	GTCCAAGAGCGCAGGTCCspT^spC	0	1191,2	-	-	1534,5	1509,4
67	HLAA_4533_1r20	GTCCAGGAGCTCAGGTCCspT^spC	0	1191,2	-	-	1534,5	1509,4
68	HLAA_5021_2r20	GGCCGYCTCCCACTTGTspGspC^spT	0	1463,4	-	-	-	1781,6
69	HLAA_5022_2r20	GGCYGCCTCCCACTTGCspGspC^spT	0	1448,4	-	1751,6	1791,7	1766,6
70	HLAA_5023_2r20	CGGAGTCTCCCACTTGCspGspC^spT	0	1448,4	-	1751,6	1791,7	1766,6
71	HLAA_5024_2r20	GGCCGCCTCCCACTTGCspGspC^spC	-14	1419,4	-	-	-	1737,6
72	HLAA_5271_1f20	AGTGGGAGACTCCGCCCAspT^spG	0	1255,3	1582,6	1558,5	-	1573,5
73	HLAA_5272_1f20	CAAGTGGGAGGCGGYCCAspT^spG	0	1255,3	1582,6	1558,5	-	1573,5
74	HLAA_5273_1f20	CAAGTGGGAGRCGGCCCAspT^spG	0	1255,3	1582,6	1558,5	-	1573,5
75	HLAA_5274_1f20	CAAGTGGGAGGCGGCCCTspT^spG	0	1246,3	-	-	-	1564,5
76	HLAA_5275_1f20	CAAGTGGGAGGCGGCCCGspT^spT	0	1246,3	-	-	1589,6	-
77	HLAA_5276_1f20	CAAGTGGGAGGCGGCCCGspT^spC	0	1231,3	-	-	1574,5	-
78	HLAA_5277_1f20	CAAGTGGGAGGCGGCCMGspT^spG	0	1271,3	1598,6	-	-	1589,5
79	HLAA_5278_1f20	CAAGTGGGAGGCRGCCCGspT^spG	0	1271,3	1598,6	-	-	1589,5
80	HLAA_5391_1f19	GCCCRTGAGGCGGAGCAspG^spC	0	1138,1	1465,4	-	1481,4	1456,3
81	HLAA_5392_1f19	GYCCATGCGGCGGAGCAspG^spC	0	1138,1	1465,4	-	1481,4	1456,3
82	HLAA_5393_1f19	GCCCGTCGGGCGGAGCAspG^spC	0	1138,1	1465,4	-	1481,4	1456,3
83	HLAA_5394_1f19	GCCCATGTGGCGGAGCAspG^spC	0	1138,1	1465,4	-	1481,4	1456,3
84	HLAA_5395_1f19	GTCCATGCGGCGGAGCAspG^spT	0	1153,1	-	-	1496,4	1471,3
85	HLAA_5396_1f19	GCCCGTYGGGCGGAGCAspG^spT	0	1153,1	-	-	1496,4	1471,3
86	HLAA_5397_1f19	GCCCATGAGGCGGAGCAspG^spT	0	1153,1	-	-	1496,4	1471,3
87	HLAA_5398_1f19	GCCCWTGTGGCGGAGCAspG^spT	0	1153,1	-	-	1496,4	1471,3
88	HLAA_5399_1f19	GCCMGTGTGGCGGAGCAspG^spT	0	1153,1	-	-	1496,4	1471,3
89	HLAA_5591_1r20	GCGGAGCCACTCCACGCAspC^spT	0	1113,1	-	1416,3	-	-
90	HLAA_5592_1r20	GCGGAGCCCGTCCACGCAspC^spT	0	1113,1	-	1416,3	-	-
91	HLAA_5593_1r20	GCGGAGCCACTCCACGCAspC^spA	0	1122,1	-	-	1465,4	-
92	HLAA_5594_1r20	GCGGAGCCCGTCCACTCAspC^spG	0	1138,1	-	-	-	1456,3
93	HLAA_5595_1r20	GCGGAGCCAGTCCACGCAspC^spG	0	1138,1	-	-	-	1456,3
94	HLAA_5596_1r20	GCGGAGCCMGTCCACGCAspC^spG	0	1138,1	-	-	-	1456,3
95	HLAA_5597_1r20	GCGGAGCCACTCCACGCAspC^spC	0	1098,1	1425,4	-	1441,4	-
96	HLAA_5598_1r20	GCGGAGCCCGTCCACGCAspC^spC	0	1098,1	1425,4	-	1441,4	-
	HLAA_5599_1r20	GCGGAGCCACTCCACGCAspG^spG	0	1178,1	-	-	-	1496,3
97	HLAA_5711_2f20	TGGAGGGCCKGTGCGTGspGspA^spG	0	1537,4	-	-	-	1855,6
98	HLAA_5712_2f20	TGGAGGGYGAGTGCGTGspGspA^spG	0	1537,4	-	-	-	1855,6
99	HLAA_5713_2f20	TGSAGGGCCGGTGCGTGspGspA^spG	0	1537,4	-	-	-	1855,6
100	HLAA_5714_2f20	TGGATGSCACGTGCGTGspGspA^spG	0	1537,4	-	-	-	1855,6
101	HLAA_5715_2f20	TGGAGGGCACSTGCGTGspGspA^spG	0	1537,4	-	-	-	1855,6
102	HLAA_5716_2f20	TGGAGGGCACGTGMGTGspGspA^spC	0	1497,4	-	-	1840,7	1815,6
103	HLAA_5717_2f20	TGGAGGGCYGGTGCGTGspGspA^spC	0	1497,4	-	-	1840,7	1815,6

TABLE V

No	Name	Sequence	CT	Primer Masses	A	C	G	T
1	HLAB_971_2f20	CCCACTCCATGAGGCATspTspT^spC	0	1540,3	-	1843,7	1883,8	1858,7
2	HLAB_972_2f20	CCCACTYCATGAGGTATspTspT^spC	0	1540,3	-	1843,7	1883,8	1858,7
3	HLAB_2061_1f20	CGACGCCGCGAGTCMGAGspG^spA	-28	1150,1	1477,4	1453,3	-	1468,3
4	HLAB_2062_1f20	CGACGCCACGAGTCCGAGspG^spA	-28	1150,1	1477,4	1453,3	-	1468,3
5	HLAB_2063_1f20	CGACGCCGCGAGTCCRAGspA^spG	0	1178,1	1505,4	-	1521,4	-
6	HLAB_2064_1f20	CGACGCCRCGAGTCCGAGspA^spG	0	1178,1	1505,4	-	1521,4	-
7	HLAB_2221_1r19	GCCCCTCCTGCTCCACCspC^spA	0	1098,3	1425,4	-	1441,4	-
8	HLAB_2222_1r19	GCCCCTCYTGCTCTATCspC^spA	0	1098,3	1425,4	-	1441,4	-
9	HLAB_2591_2f20	GGCCGGAGTATTGGGACspGspG^spG	0	1513,4	-	-	1856,7	-
10	HLAB_2592_2f20	GGCCGGAGTATTGGGACspGspA^spG	0	1497,4	-	-	1840,7	-
11	HLAB_2593_2f20	GGCCGGAGTATTGGGACspCspC^spG	-28	1405,4	-	-	1748,7	-
12	HLAB_2594_2f20	GGCCGGAGTATTGGGATspCspG^spG	0	1488,4	1815,7	-	1831,7	-
13	HLAB_2595_2f20	GGCCGGAGTTTTGGGACspCspG^spG	-28	1445,4	1772,7	-	1788,7	-
14	HLAB_2596_2f20	GGCCGGAGCATTGGGACspCspG^spG	-28	1445,4	1772,7	-	1788,7	-
15	HLAB_2597_2f20	GGCCGGGATATTGGGACspCspG^spG	-28	1445,4	1772,7	-	1788,7	-
16	HLAB_2598_2f20	GGCCRGAATATTGGGACspCspG^spG	-28	1445,4	1772,7	-	1788,7	-
17	HLAB_2599_2f20	GGCGGGMGTATTGGGACspCspG^spG	-28	1445,4	1772,7	-	1788,7	-
18	HLAB_25910_2f20	GGCCTTAGTATTGGGACspCspG^spG	-28	1445,4	1772,7	-	1788,7	-
19	HLAB_2721_1f20	GGACSGGGAGACACGGAAspC^spA	0	1122,1	-	-	-	1440,3
20	HLAB_2722_1f20	GGACGRGGAGACACGGAAspC^spA	0	1122,1	-	-	-	1440,3
21	HLAB_2723_1f20	GGACCGGAACACACAGAAspC^spT	0	1113,1	-	-	1456,4	-
22	HLAB_2724_1f20	GGACCGGAACACACAGACspC^spT	-14	1075,1	-	-	-	1393,3
23	HLAB_2725_1f20	GGACCGGGAGACACAGAAspG^spT	0	1153,1	1480,4	-	-	-
24	HLAB_2726_1f20	GGACCGGGAGATACAGATspC^spT	0	1104,1	1431,4	1407,3	1447,4	1422,3
25	HLAB_2727_1f20	GGACCGGGASACACAGATspC^spT	0	1104,1	1431,4	1407,3	1447,4	1422,3
26	HLAB_2728_1f20	GGACCGGGACACACAGATspC^spT	0	1104,1	1431,4	1407,3	1447,4	1422,3
27	HLAB_2729_1f20	GGACCSGGAGACACAGATspC^spT	0	1104,1	1431,4	1407,3	1447,4	1422,3
28	HLAB_2921_2f19	CAAGACCAACACACAGspGspC^spT	0	1458,3	-	-	1801,6	-
29	HLAB_2922_2f19	CAAGSCCCAGGCACAGspGspC^spT	0	1458,3	-	-	1801,6	-
30	HLAB_2923_2f19	CAAGACCAACACACGGspAspC^spT	-28	1414,3	-	-	1757,6	1732,5
31	HLAB_2924_2f19	GAAGGCCTCCGCGCAGspAspC^spT	-28	1414,3	-	-	1757,6	1732,5
32	HLAB_2925_2f19	CAAGGCCMAGGCACAGspAspC^spT	-28	1414,3	-	-	1757,6	1732,5
33	HLAB_2926_2f19	CAAGSGCCAGGCACAGspAspC^spT	-28	1414,3	-	-	1757,6	1732,5
34	HLAB_2927_2f19	GAAGACCAACACACAGspAspC^spT	-28	1414,3	-	-	1757,6	1732,5
35	HLAB_3021_2f19	GCACAGACTGACCGAGspTspG^spG	0	1528,4	-	-	1871,7	-
36	HLAB_30211_2f19	ACACAGACTTACAGAGspAspG^spA	-28	1493,5	1820,8	-	1836,8	-
37	HLAB_3022_2f19	ACACAGACTTACCGAGspAspG^spG	0	1537,4	1864,7	-	-	-
38	HLAB_3023_2f19	RCACAGACTGACCGAGspAspG^spG	0	1537,4	1864,7	-	-	-
39	HLAB_3024_2f19	GCACAGACTGGCCGAGspTspG^spA	-28	1481,4	1811,7	-	1827,7	-
40	HLAB_3025_2f19	ACACAGACTTACCGAGspTspG^spA	-28	1481,4	1811,7	-	1827,7	-
41	HLAB_3026_2f19	RCACAGACTGACCGAGspTspG^spA	-28	1481,4	1811,7	-	1827,7	-
42	HLAB_3027_2f19	ACACAGGCTGACCGAGspAspG^spA	-28	1493,5	1820,8	-	1836,8	-
43	HLAB_3028_2f19	RCACAGACTGACCGAGspAspG^spA	-28	1493,5	1820,8	-	1836,8	-
44	HLAB_3029_2f19	GCRCAGACTTACCGAGspAspG^spA	-28	1493,5	1820,8	-	1836,8	-
45	HLAB_30210_2f19	ACACRGACTTACCGAGspAspG^spA	-28	1493,5	1820,8	-	1836,8	-
46	HLAB_3621_2f20	CGGGTCTCACACCCTCCspAspC^spA	-28	1413,4	-	-	1756,7	-
47	HLAB_3622_2f20	CGGGTCTCACAYCATCCspAspG^spA	-14	1467,4	1794,7	1770,6	1810,7	1785,6
48	HLAB_3623_2f20	CGGKTCTCACACCCTCCspAspG^spA	-14	1467,4	1794,7	1770,6	1810,7	1785,6
49	HLAB_3624_2f20	CGGGTCTCACACTTGGCspAspG^spA	-14	1467,4	1794,7	1770,6	1810,7	1785,6
50	HLAB_3625_2f20	CGGGTCTCACATCATCCspAspG^spG	-14	1483,4	-	-	-	1801,6
51	HLAB_3626_2f20	CGGGTCTCACACCCTCCspAspG^spT	0	1472,4	-	-	1815,7	-
52	HLAB_3631_1r20	CCCASGTCGCAGCCGTACspA^spT	-28	1085,1	-	1388,3	1428,4	1403,3
53	HLAB_3632_1r20	CCCABGTCGCAGCCATACspA^spT	-28	1085,1	-	1388,3	1428,4	1403,3
54	HLAB_3633_1r20	CCCASGTCGCAGCCAAACspA^spT	-28	1085,1	-	1388,3	1428,4	1403,3

55	HLAB_3634_1r20	CCCACGTCGCAGCCAGACspA^spT	-28	1085,1	-	1388,3	1428,4	1403,3
56	HLAB_3635_1r20	CCCACGTCGCAGCCGCACspA^spT	-28	1085,1	-	1388,3	1428,4	1403,3
57	HLAB_3636_1r20	CCCACGTCGCAGCCTTACspA^spT	-28	1085,1	-	1388,3	1428,4	1403,3
58	HLAB_3637_1r20	CCCACGTCGCAGCCGTACspG^spT	0	1129,1	-	1432,3	1472,4	1447,3
59	HLAB_3691_1f20	TCCGGCCCCAKGTCGCAGspC^spC	0	1114,1	1441,4	-	1457,4	1432,3
60	HLAB_3692_1f20	TCGGGCCCCASGTCGCAGspC^spC	0	1114,1	1441,4	-	1457,4	1432,3
55	HLAB_4121_2f20	GGCGCCTCCTCCGCGGGspTspA^spC	-28	1444,4	-	1747,6	-	-
56	HLAB_4122_2f20	GGCGCCTCCTCCSCGGGspCspA^spT	0	1472,4	1799,7	-	1815,7	-
57	HLAB_4123_2f20	GGCGCYTCCTCCGCGGGspCspA^spT	0	1472,4	1799,7	-	1815,7	-
58	HLAB_4124_2f20	GGCGTCTCCTCCGCGGTspTspA^spT	0	1462,4	-	1765,6	-	-
59	HLAB_4125_2f20	GGCGCCTCCTCCGCGGGspTspA^spT	-14	1473,4	-	1776,6	-	-
60	HLAB_4181_2f20	TCCTCCGCGGGTATGAAspCspA^spG	0	1481,4	1808,7	-	-	-
61	HLAB_4182_2f20	TCCTCCACGGGTACCACspCspA^spG	0	1457,4	-	-	-	1775,6
62	HLAB_4183_2f20	TCCTGCGCGGGTACCACspCspA^spG	0	1457,4	-	-	-	1775,6
63	HLAB_4184_2f20	TCCTCCGCGGGTACCACspCspA^spG	0	1457,4	-	-	-	1775,6
64	HLAB_4185_2f20	TCCTCTGCGGGTACCACspCspA^spG	0	1457,4	-	-	-	1775,6
65	HLAB_4186_2f20	TCCTCCGCGGGTACCAGspCspA^spG	0	1497,4	1824,7	1800,6	1840,7	1815,6
66	HLAB_4187_2f20	TMCTCCGCGGGTACCGGspCspA^spG	0	1497,4	1824,7	1800,6	1840,7	1815,6
67	HLAB_4188_2f20	TCCTCCGCGGGTACCAGspCspG^spG	0	1513,4	-	-	1856,7	-
68	HLAB_4191_2r20	AATCCTTGCCGTCGTAGspGspC^spT	-14	1474,4	1801,7	-	-	-
69	HLAB_4192_2r20	AATCCTTGCCGTCGTAGspGspC^spA	-28	1469,4	-	-	1812,7	-
70	HLAB_4193_2r20	AATTCTTGCCGTCGTAGspGspC^spG	0	1513,4	1840,7	-	1856,7	1831,6
71	HLAB_4194_2r20	AATCTTTGCCGTCGTAGspGspC^spG	0	1513,4	1840,7	-	1856,7	1831,6
72	HLAB_4195_2r20	AATCCTTGCCGTCGYAGspGspC^spG	0	1513,4	1840,7	-	1856,7	1831,6
73	HLAB_4351n_1r20	TCMTTCAGGGCGATGTAAspT^spC	-14	1201,3	-	1504,4	-	1519,4
74	HLAB_4352n_1r20	TCGTTCAGGGCGATGTAAspT^spT	0	1230,3	-	1533,5	-	-
75	HLAB_5271_1f20	CAAGTGGGAGGCGGCCCTspT^spG	0	1246,3	-	-	-	1564,5
76	HLAB_5272_1f20	CAAGTKGGAGGCGGCCCGspT^spG	0	1271,3	1598,6	1574,3	-	1589,5
77	HLAB_5391_1f20	GGCCCGTGYGGCGGAGCAspG^spC	0	1138,1	-	-	1481,3	1456,3
78	HLAB_5392_1f20	GGCCCGTGTCGCGGAGCAspG^spG	0	1178,1	1505,4	-	-	-
79	HLAB_5393_1f20	GGCCCGTGWGGCGGAGCAspG^spG	0	1178,1	1505,4	-	-	-
80	HLAB_5394_1f20	GGCCCGTGAGGCGGAGCAspG^spT	0	1153,1	-	-	1496,4	-
81	HLAB_5591_1r20	GCGGAGCGACTCCACGCAspC^spT	0	1113,1	-	-	1456,4	-
82	HLAB_5592_1r20	GCGGAGCCACTCCACGCAspC^spT	0	1113,1	-	-	1456,4	-
83	HLAB_5593_1r20	GCGGAGCCAATCCACGCAspC^spT	0	1113,1	-	-	1456,4	-
84	HLAB_5594_1r20	GCGGAGCCACTCCACGCAspC^spG	0	1152,1	-	-	-	1470,3
85	HLAB_5595_1r20	GCGGAGCGACTCCRCGCAspC^spA	-14	1122,1	1449,1	1425,3	-	-
86	HLAB_5596_1r20	GCGGAGCSACTCCACGCAspC^spA	-14	1122,1	1449,1	1425,3	-	-
87	HLAB_5597_1r20	GCGGAGCCCGTCCACGCAspC^spA	-14	1122,1	1449,1	1425,3	-	-
88	HLAB_5711_1r20	CTCCAGGTAYCTGCGGAGspC^spG	0	1154,1	1481,4	-	-	-
89	HLAB_5712_1r20	CTCCAGGTRTCTGCGGAGspC^spC	0	1114,1	1441,4	1417,3	-	-
90	HLAB_583_1r19	ACCTGGAGAACGGGAAGspG^spA	0	1178,1	1505,4	-	1521,4	-

TABLE VI

No	Name	Sequence	CT	Masses Primer	A	C	G	T
1	DRB1_1251_1r20	CATTGAAGAAATGACACTspC^spC	0	1098,1	-	1392,3	-	-
2	DRB1_1252_1r20	CGTTGAAGAAATGACACTspT^spA	0	1230,1	-	-	-	1548,5
3	DRB1_1253_1r20	CATTGAAGAAATGACATTspC^spA	0	1113,1	1440,4	1416,3	1456,4	1431,3
4	DRB1_1254_1r20	CATTGAAGAAWTAACACTspC^spA	0	1113,2	1440,4	1416,3	1456,4	1431,3
5	DRB1_1255_1r20	CRTTGAAGAAATGACACTspC^spA	0	1113,3	1440,4	1416,3	1456,4	1431,3
6	DRB1_1961_1f19	CATCTATAACCAAGAGGspA^spA	0	1162,1	-	-	-	1480,3
7	DRB1_1962_1f19	CTTCTATCACCAAGARGspA^spG	0	1178,1	1505,4	-	-	1496,3
8	DRB1_1963_1f19	CTTCTATAATCARGAGGspA^spG	0	1178,1	1505,4	-	-	1496,3
9	DRB1_1964_1f19	CGTCCATAACCAAGAGGspA^spG	0	1178,1	1505,4	-	-	1496,3
10	DRB1_1965_1f19	CATCTATAACCAAGAGGspA^spG	0	1178,1	1505,4	-	-	1496,3
11	DRB1_1966_1f19	CTTCCATAACCRGGAGGspA^spG	0	1178,1	1505,4	-	-	1496,3
12	DRB1_1967_1f19	CTTCGATAACCAGGAGGspA^spG	0	1178,1	1505,4	-	-	1496,3
13	DRB1_1968_1f19	CTTCTATAACCTGGAGGspA^spG	0	1178,1	1505,4	-	-	1496,3
14	DRB1_1971_1r20	CGTCGCTGTCGAAGCGCAspG^spG	0	1178,1	1505,4	-	-	1496,3
15	DRB1_1972_1r20	CGTCGCTGTCGTAGCGCGspC^spG	0	1154,1	-	-	-	1472,3
16	DRB1_1973_1r20	CGTCGCTGTCGAAGCGCAspA^spG	0	1162,1	-	-	-	1480,3
17	DRB1_1974_1r20	CGTCGCTGTCGAAGYGCAspC^spG	-28	1110,1	1437,4	-	1453,4	1428,3
18	DRB1_1975_1r20	CGTCGCTGTCGAASCGCAspC^spG	-28	1110,1	1437,4	-	1453,4	1428,3
19	DRB1_2271_1f20	CGACAGCGACGTGGGGGAspC^spT	0	1113,1	1440,4	-	-	-
20	DRB1_2272_1f20	CGACAGCGACGTGVGGGAspG^spT	0	1153,1	1480,4	-	-	1471,3
21	DRB1_2611_1r20	TTCTGGCTGTTCCAGTACspT^spG	0	1231,2	-	-	1574,5	-
22	DRB1_2612_1r20	TTCTGGCTGTTCCAGTACspC^spC	0	1074,1	-	1377,3	-	-
23	DRB1_2613_1r20	TTCTGGCTGTTCCAGTAGspT^spC	0	1231,2	-	1534,4	-	-
24	DRB1_2614_1r20	TTCTGGCTGTTCCAGTRCspT^spC	-14	1177,2	1504,5	1480,4	1520,5	-
25	DRB1_2615_1r20	TTCYGGCTGTTCCAGGACspT^spC	-14	1177,2	1504,5	1480,4	1520,5	-
26	DRB1_2861_1f19	CTGGAACAGCCAGAAGAspA^spC	-28	1122,1	1449,4	-	-	-
27	DRB1_2862_1f19	CTGGAACAGCCRGAAGGspA^spC	0	1138,1	1465,4	1441,3	-	1456,3
28	DRB1_2991_1f20	GAAGGACHTCCTGGAGCAspG^spG	0	1178,1	-	1481,3	-	-
29	DRB1_2992_1f20	GAAGGACATCCTGGGAGAspC^spA	-14	1108,1	1435,1	-	1451,4	-
30	DRB1_2993_1f20	GAAGGACATCCTGGARGAspC^spA	-14	1108,1	1435,1	-	1452,4	-
31	DRB1_2994_1f20	GAAGGACYTCCTGGAAGAspC^spA	-14	1108,1	1435,1	-	1453,4	-
32	DRB1_2995_1f20	GAAGGACATCCTGGAGCAspG^spA	0	1162,1	1489,4	-	1505,4	-
33	DRB1_2996_1f20	GAAGGACHTCCTGGAGCGspG^spA	0	1178,1	-	-	1521,4	-
34	DRB1_2997_1f20	GAAGGACHTCCTGGAAGAspC^spG	0	1138,1	1465,4	-	-	-
35	DBR1_3081_1r20	GTCTGCAATAGGTGTCCAspC^spG	0	1138,1	-	1441,3	-	-
36	DRB1_3082_1r20	GTCTGCARTAGGCGTCCAspC^spC	-14	1084,1	1411,4	1387,3	1427,4	1402,3
37	DRB1_3083_1r20	GTCTGCAGTAATTGTCCAspC^spC	-14	1084,1	1411,4	1387,3	1427,4	1402,3
38	DRB1_3084_1r20	GTCTGCACACGGTGTCCAspC^spC	-14	1084,1	1411,4	1387,3	1427,4	1402,3
39	DRB1_3085_1r20	GTCTGCAGTAGGTGTCCAspC^spC	-14	1084,1	1411,4	1387,3	1427,4	1402,3
40	DRB1_3086_1r20	GTCTGCAATAGGTGTCCAspC^spC	-14	1084,1	1411,4	1387,3	1427,4	1402,3
41	DRB1_341_1f19	TGCAGACACAACTACSGspG^spG	0	1194,1	-	1497,3	-	1512,3
42	DRB1_3451_1r20	CGCTGCACTGTGAATCTCspT^spC	0	1191,3	1518,5	1494,4	-	-
43	DRB1_3452_1r20	CTCTGCACTGTGAAGCTCspT^spC	0	1191,3	1518,5	1494,4	-	-
44	DRB1_3453_1r20	CGCTGCACYGTGAAGCTCspT^spC	0	1191,3	1518,5	1494,4	-	-

The resolution achievable by 19 markers each for HLA-A and HLA-B and the ten markers for HLA-DRB1 are listed in Tables VII to IX below.

TABLE VII

Frequent Alleles of HLA-A	Group of frequent Alleles with same four-digit type	Rare Alleles with same Mini-Haplotype Profile	Resolution (in %)
A*0101	A*010101, A*010102	A*0103, A*0104N, A*0109	98,3
A*0201	A*02010101, A*020101021, A*020103, A*020104, A*020108, A*020109	A*0204, A*0209, A*0225, A*0231, A*0232N, A*0242, A*0243N, A*0253N, A*0258, A*0260, A*0264, A*0266, A*0267	93,4
	A*020102		100
	A*020105		100
	A*020106		100
	A*020107		100
A*0301	A*03010101, A*03010102N	A*0303N, A*0304, A*0305, A*0306, A*0311N	97,6
	A*030102		100
	A*030103		100
A*2301	A*2301	A*2306, A*2307N, A*2308N	98,6
A*2402	A*24020101, A*240201021, A*240202, A*240203, A*240204	A*2404, A*2409N, A*2411N, A*2426, A*2427, A*2432, A*2435, A*2436N, A*2437, A*2439	94,5
A*2902	A*290201	A*29010101, A*29010102N, A*2906, A*2908N	98,3
	A*290202		100
A*3001	A*3001		100
A*3002	A*3002		100

Capture: Alleles in a same field have the same mini-haplotype profile; grey high lighted are all alleles with identical sequences over exons 2 and 3.

TABLE VIII

Frequent Alleles of HLA-B	Groups of frequent Alleles with same four-digit type	Rare Alleles with same Mini-Haplotype Profile	Resolution (in %)
B*0702	B*070201, B*070202, B*070203, B*070204	B*0703, B*0721, B*0722, B*0723, B*0730, B*0733, B*0735	98,0
B*0801	B*0801	B*0808N, B*0818, B*0819N	99,3
B*1302	B*1302	B*1308	99,6
B*1501	B*15010101, B*15010102N, B*150103, B*150104	B*1528, B*1533, B*1534, B*1560, B*1575, B*1578, B*1579N, B*1581, B*1582	97,6
	B*150102		100
B*1801	B*180101, B*180102	B*1805, B*1817N	99,3
B*3501	B*350101, B*350102	B*3507, B*3540N, B*3541, B*3542, B*5305	98,7
B*3503	B*3503	B*3536	99,6
B*4001	B*400101, B*400102,	B*4011, B*401401, B*401402, B*401403, B*4022N	98,7
	B*400103		100
	B*400104	B*4004	99,6
B*4402	B*44020101, B*44020102S, B*440202, B*440203	B*4411, B*4419N, B*4422, B*4423N, B*4427, B*4433, B*4434, B*4435	97,8
B*4403	B*440301	B*4413, B*4426, B*4429, B*4430, B*4432, B*4436, B*4437, B*4438, B*4439	98,2
	B*440302	B*4407	99,6
B*5101	B*510101, B*510102, B*510105	B*5111N, B*5112, B*5114, B*5118, B*5126, B*5127N, B*5128, B*5130, B*5132, B*5133	97,6
	B*510103		100
	B*510104	B*5124	99,6
B*5701	B*570101	B*5706, B*5708	99,5
	B*570102		100

Capture: Alleles in a same field have the same mini-haplotype profile; grey high lighted are all alleles with identical sequences over exons 2 and 3.

TABLE IX

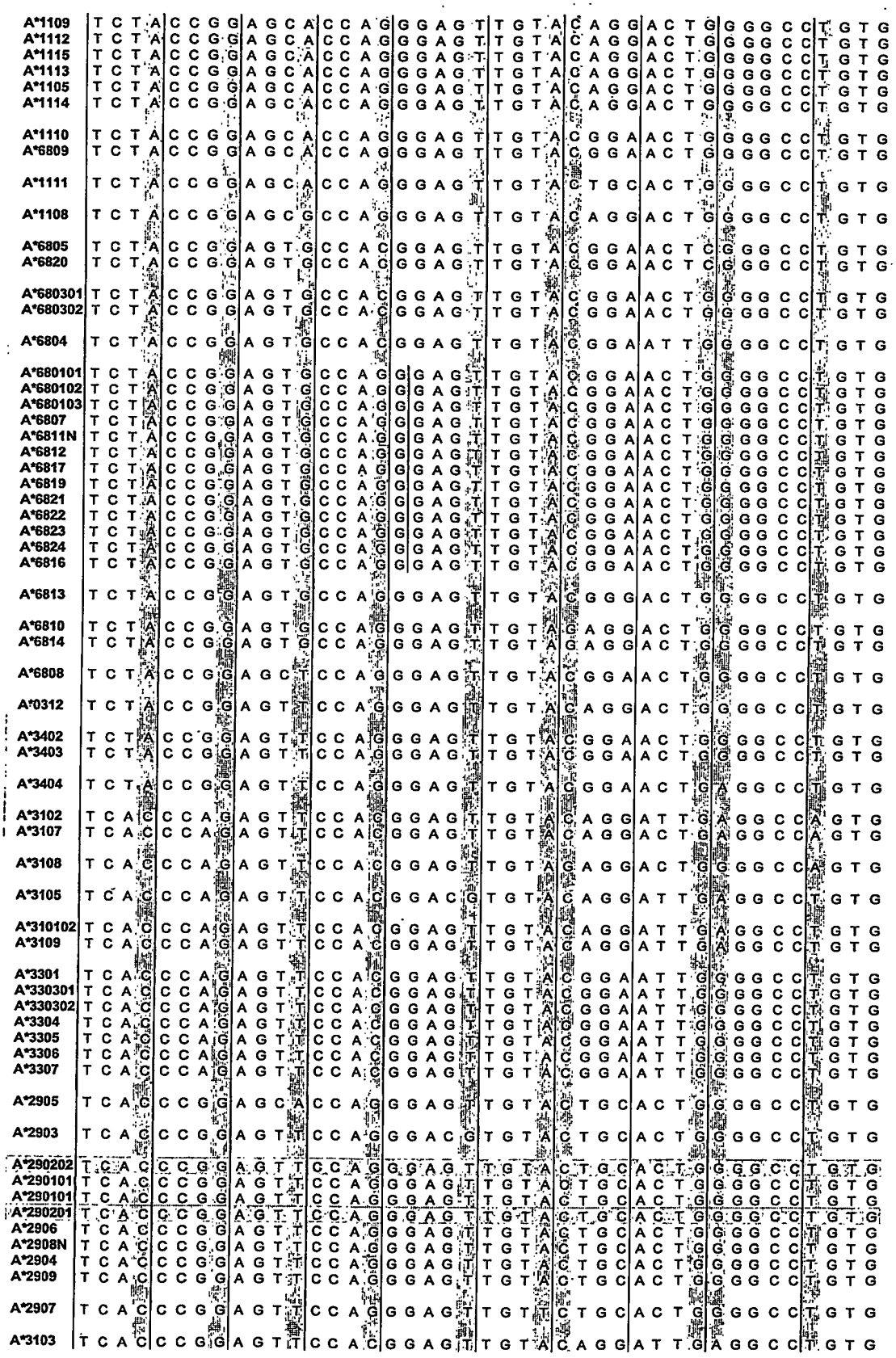
Frequent Alleles of HLA-DRB1*	Groups of frequent Alleles with same four-digit type	Rare Alleles with same Mini-Haplotype Profile	Resolution (in %)
DRB1*0101	DRB1*010101	DRB1*0105, DRB1*0107, DRB1*0111	98,9
	DRB1*010102		100
DRB1*0301	DRB1*030101, DRB1*030102	DRB1*0307, DRB1*0312, DRB1*0313, DRB1*0315, DRB1*0316, DRB1*0318, DRB1*0322, DRB1*0323	97,2
DRB1*0401	DRB1*040101, DRB1*040102	DRB1*0409, DRB1*0426, DRB1*0433	98,6
DRB1*0701	DRB1*070101, DRB1*070102	DRB1*0703, DRB1*0704, DRB1*0705, DRB1*0707	98,3
DRB1*1101	DRB1*110101, DRB1*110102, DRB1*110103, DRB1*110104, DRB1*110105	DRB1*112701, DRB1*112702, DRB1*1130, DRB1*1139	97,5
DRB1*1104	DRB1*110401, DRB1*110402	DRB1*1134, DRB1*1146	98,9
DRB1*1302	DRB1*130201, DRB1*130202	DRB1*1331, DRB1*1339, DRB1*1341	98,6
DRB1*1501	DRB1*150101, DRB1*150103, DRB1*150105	DRB1*1503, DRB1*1506, DRB1*1509, DRB1*1513	98,0
	DRB1*150102		100
	DRB1*150104	DRB1*1512	99,4

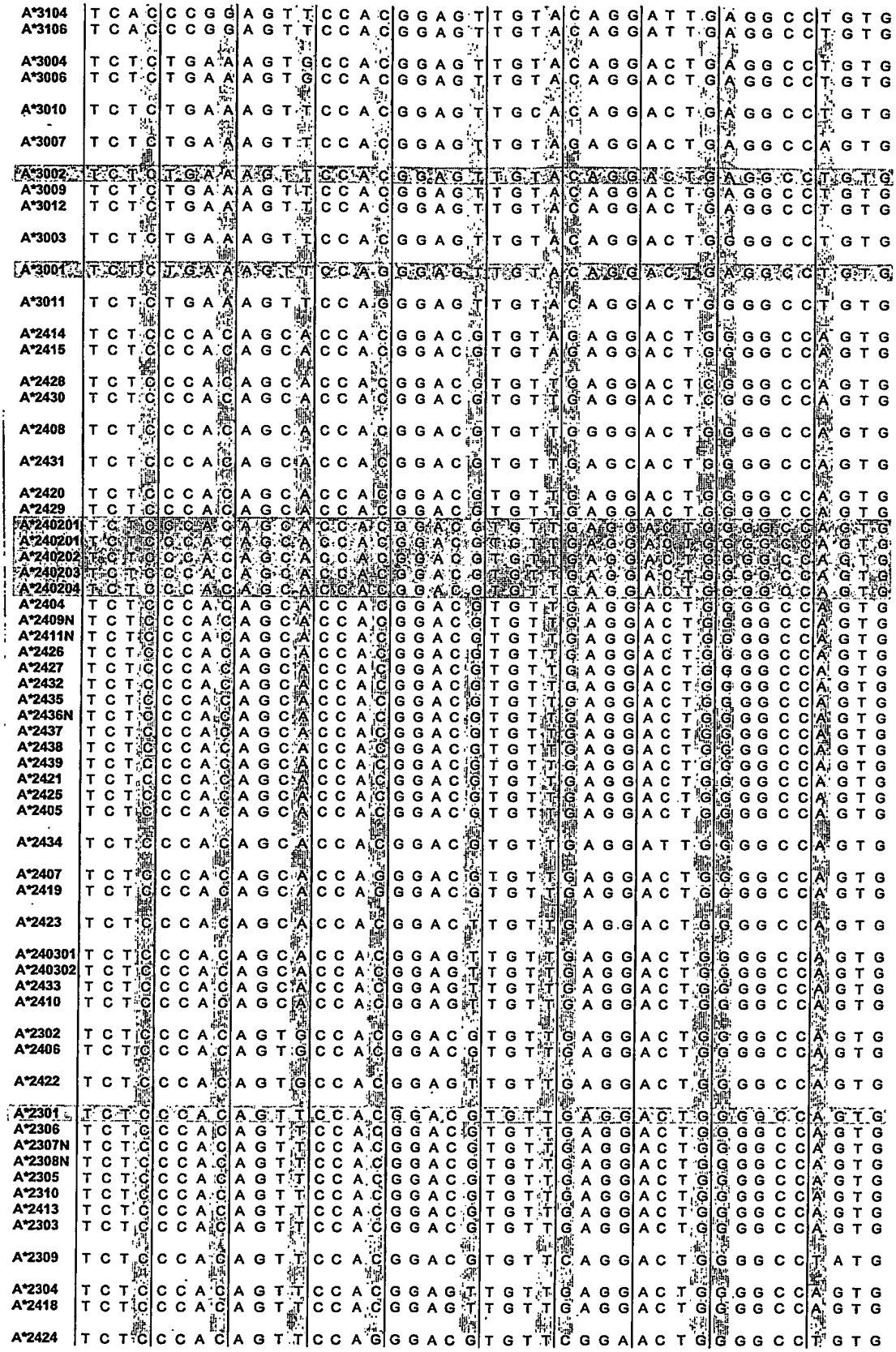
Capture: Alleles in a same field have the same mini-haplotype profile; grey high lighted are all alleles with identical sequences over exon 2 (base 101 to 356)

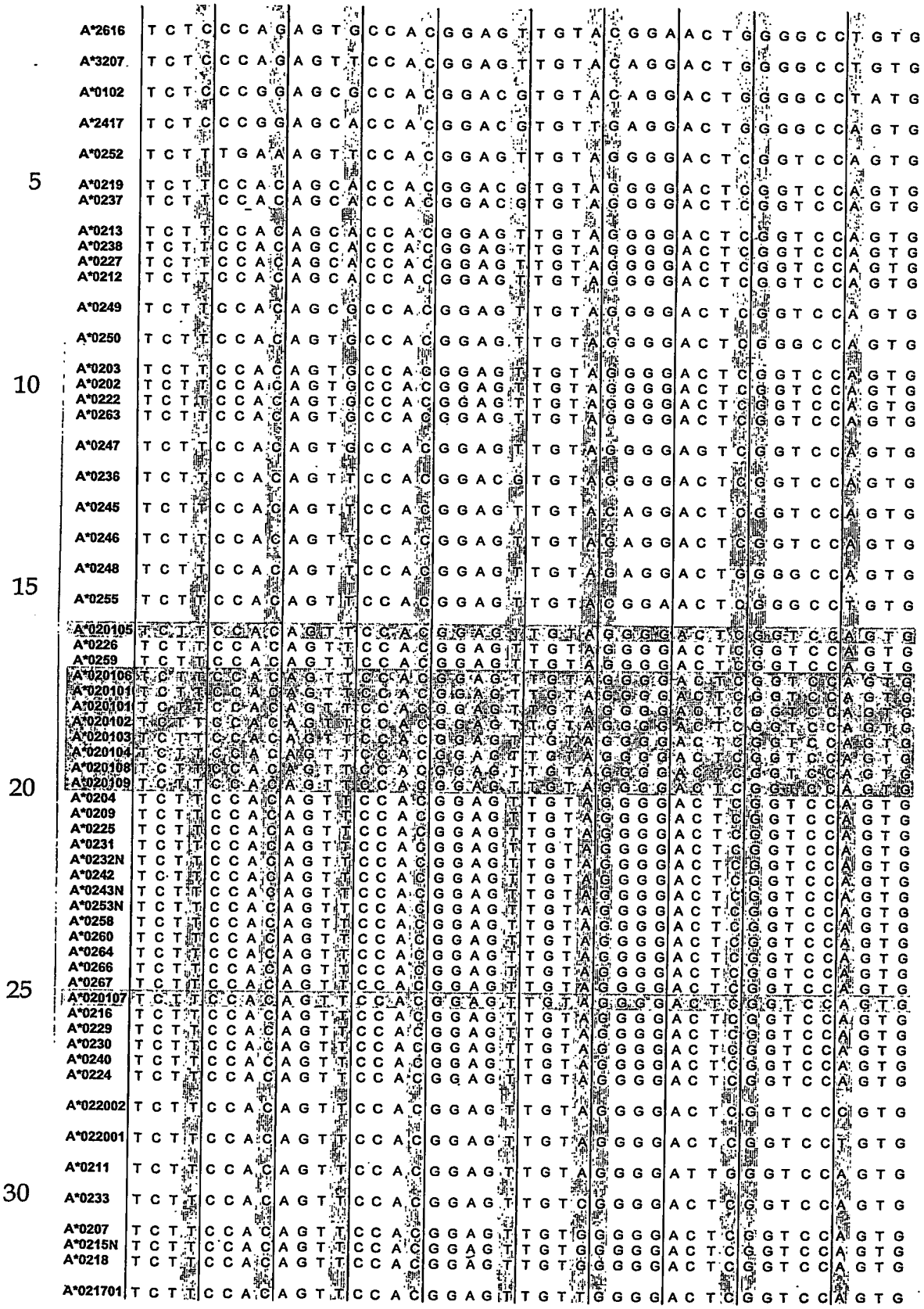
The complete list of HLA alleles and sub-groups generated by the most informative mini-haplotyping markers (ten each for HLA-A, HLA-B and HLA-DRB1) are listed in Tables X to XII below.

TABLE X

Position	95	96	97	98	411	412	413	414	536	537	538	539	279	280	281	282	567	568	569	570	571	365	366	367	368	256	257	258	259	289	290	291	292	238	239	240	241	242	270	271	272	273
cDNA																																											
A*3008	T	C	T	A	T	G	A	A	A	G	T	T	C	C	A	G	G	G	A	G	T	T	G	T	A	C	A	G	G	A	C	T	G	A	G	G	C	C	T	G	T	G
A*6806	T	C	T	A	T	G	A	A	A	G	T	G	C	C	A	G	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*0244	T	C	T	A	C	C	A	C	A	G	C	A	C	C	A	C	G	G	A	G	T	T	G	T	A	G	G	G	G	A	C	T	C	G	G	T	C	C	A	G	T	G
A*0254	T	C	T	A	C	C	A	C	A	G	C	A	C	C	A	C	G	G	A	C	G	T	G	T	A	G	G	G	G	A	C	T	C	G	G	T	C	C	A	G	T	G
A*0205	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	G	G	G	G	A	C	T	C	G	G	T	C	C	A	G	T	G
A*0208	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	G	G	G	G	A	C	T	C	G	G	T	C	C	T	G	T	G
A*6815	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	C	G	G	G	C	C	T	G	T	G
A*6802	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	G	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*6818N	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	G	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*0228	T	C	T	A	C	C	A	C	A	G	T	T	C	C	A	C	G	G	A	G	T	T	G	T	A	G	G	G	G	A	C	T	C	A	G	T	C	C	A	G	T	G
A*0206	T	C	T	A	C	C	A	C	A	G	T	T	C	C	A	C	G	G	A	G	T	T	G	T	A	G	G	G	G	A	C	T	C	G	G	T	C	C	A	G	T	G
A*0214	T	C	T	A	C	C	A	C	A	G	T	T	C	C	A	C	G	G	A	G	T	T	G	T	A	G	G	G	G	A	C	T	C	G	G	T	C	C	A	G	T	G
A*0221	T	C	T	A	C	C	A	C	A	G	T	T	C	C	A	C	G	G	A	G	T	T	G	T	A	G	G	G	G	A	C	T	C	G	G	T	C	C	A	G	T	G
A*0257	T	C	T	A	C	C	A	C	A	G	T	T	C	C	A	C	G	G	A	G	T	T	G	T	A	G	G	G	G	A	C	T	C	G	G	T	C	C	A	G	T	G
A*0251	T	C	T	A	C	C	A	C	A	G	T	T	C	C	A	C	G	G	A	G	T	T	G	T	A	G	G	G	G	A	C	T	C	G	G	T	C	C	A	G	T	G
A*0261	T	C	T	A	C	C	A	C	A	G	T	T	C	C	A	C	G	G	A	G	T	T	G	T	A	G	G	G	G	A	C	T	C	G	G	T	G	C	A	G	T	G
A*0210	T	C	T	A	C	C	A	C	A	G	T	T	C	C	A	C	G	G	A	G	T	T	G	T	A	G	G	G	G	A	C	T	C	G	G	T	C	C	A	G	T	G
A*6901	T	C	T	A	C	C	A	C	A	G	T	T	C	C	A	G	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*2504	T	C	T	A	C	C	A	C	A	G	C	A	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*2608	T	C	T	A	C	C	A	C	A	G	C	A	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*2603	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	C	G	G	G	C	C	T	G	T	G
A*2606	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	C	G	G	G	C	C	T	G	T	G
A*2610	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*2609	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*250101	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*250102	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*2601	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*2602	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*2605	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*2611N	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*2614	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*2615	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*2617	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*2604	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*6603	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*2612	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*2618	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*4301	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	C	T	G	C	A	C	T	G	G	G	G	C	C	T	G	T	G
A*260701	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	G	G	G	G	A	C	T	G	G	G	G	C	C	A	G	T	G
A*260702	T	C	T	A	C	C	A	C	A	G	T	G	C	C	A	C	G	G	A	G	T	T	G	T	A	G	G	G	G	A	C	T	G	G	G	T	C	C	A	G	T	G
A*2619	T	C	T	A	C	C	A	G	A	G	T	G	C	C	A	G	G	G	A	G	T	T	G	T	A	C	A	G	G	A	C	T	G	A	G	G	C	C	T	G	T	G
A*3401	T	C	T	A	C	C	A	G	A	G	T	G	C	C	A	G	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	A	G	T	G
A*3405	T	C	T	A	C	C	A	G	A	G	T	G	C	C	A	G	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	A	G	T	G
A*6602	T	C	T	A	C	C	A	G	A	G	T	G	C	C	A	G	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*2502	T	C	T	A	C	C	A	G	A	G	T	G	C	C	A	G	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*2613	T	C	T	A	C	C	A	G	A	G	T	G	C	C	A	G	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*6601	T	C	T	A	C	C	A	G	A	G	T	G	C	C	A	G	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*6604	T	C	T	A	C	C	A	G	A	G	T	G	C	C	A	G	G	G	A	G	T	T	G	T	A	C	G	G	A	A	C	T	G	G	G	G	C	C	T	G	T	G
A*0241	T	C	T	A	C	C	A	G	A	G	T	T	C	C	A	C	G	G	A	G	T	T	G	T	A	G	G	G	G	A	C	T	C	G	G	T	C	C	A	G	T	G
A*1106	T	C	T	A	C	C	G	G	A	G	C	A	C	C	A	G	G	G	A	G	T	T	G	T	A	C	A	G	G	A	C	T	C	G	G	G	C	C	T	G	T	G
A*1103	T	C	T	A	C	C	G	G	A	G	C	A	C	C	A	G	G	G	A	G	T	T	G	T	A	C	A	G	G	A	C	T	G	G	G	G	C	C	T	G	T	G
A*1104	T	C	T	A	C	C	G	G	A	G	C	A	C	C	A	G	G	G	A	G	T	T	G	T	A	C	A	G	G	A	C	T	G	G	G	G	C	C	T	G	T	G
A*1107	T	C	T	A	C	C	G	G	A	G	C	A	C	C	A	G	G	G	A	G	T	T	G	T	A	C	A	G	G	A	C	T	G	G	G	G	C	C	T	G	T	G
A*110101	T	C	T	A	C	C	G	G	A	G	C	A	C	C	A	G	G	G	A	G	T	T	G	T	A	C	A	G	G	A	C	T	G	G	G	G	C	C	T	G	T	G
A*110102	T	C	T	A	C	C	G	G	A	G	C	A	C	C	A	G	G	G	A	G	T	T	G	T	A	C	A	G	G	A	C	T	G	G	G	G	C	C	T	G	T	G
A*1102	T	C	T	A	C	C	G	G	A	G	C	A	C	C	A	G	G	G	A	G	T	T	G	T	A	C	A	G	G	A	C	T	G	G	G	G	C	C	T	G	T	G







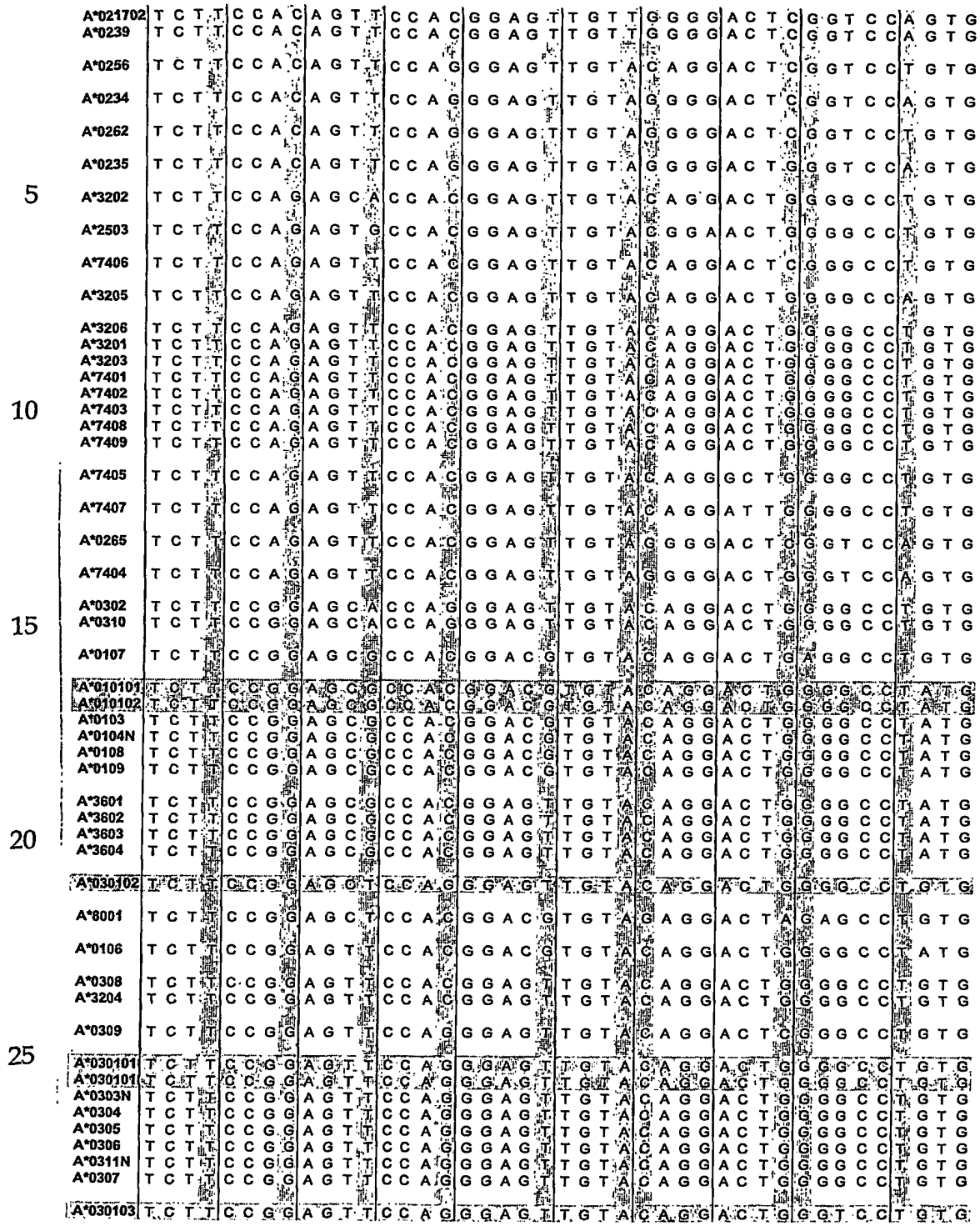


TABLE XI

Position	536	537	538	539	419	420	421	422	423	559	560	561	562	408	409	410	411	412	269	270	271	272	358	359	360	361	362	298	299	300	301	302	363	364	365	366	203	204	205	206	366	367	368	369
cDNA																																													
B-0804	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	C	C	A	G	A	G	G	A	G	A	G	C	A	T	G	G	A	G	A	G	T	A	C
B-0817	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	C	C	A	G	A	G	G	A	G	A	A	C	A	T	G	G	A	G	A	G	T	A	C
B-4102	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	C	C	A	G	A	G	G	A	G	A	G	C	A	T	G	G	G	A	A	G	T	A	C
B-4103	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	C	C	A	G	A	G	G	A	G	A	G	G	A	T	G	G	G	A	A	G	T	A	C
B-4101	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	C	C	A	G	A	G	G	A	G	A	G	G	A	T	G	G	G	A	A	G	T	A	T
B-4105	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	C	C	A	G	A	G	G	A	G	A	G	G	A	T	G	G	G	A	A	G	T	A	T
B-4106	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	C	C	A	G	A	G	G	A	G	A	G	G	A	T	G	G	G	A	A	G	T	A	T
B-0805	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	C	C	T	T	C	A	G	A	G	G	A	G	A	G	C	A	T	G	G	A	G	A	G	T	A	C
B-0809	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	T	C	A	G	A	C	G	A	G	A	G	G	A	T	G	G	A	G	A	G	T	A	T
B-0802	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	T	C	A	G	A	G	G	A	G	A	A	C	A	T	G	G	A	G	A	G	T	A	C
B-0803	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	T	C	A	G	A	G	G	A	G	A	A	C	A	T	G	G	A	G	A	G	T	A	C
B-0801	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	T	C	A	G	A	G	G	A	G	A	G	C	A	T	G	G	A	G	A	G	T	A	C
B-0808N	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	T	C	A	G	A	G	G	A	G	A	G	C	A	T	G	G	A	G	A	G	T	A	C
B-0810	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	T	C	A	G	A	G	G	A	G	A	G	C	A	T	G	G	A	G	A	G	T	A	C
B-0818	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	T	C	A	G	A	G	G	A	G	A	G	C	A	T	G	G	A	G	A	G	T	A	C
B-0819N	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	T	C	A	G	A	G	G	A	G	A	G	C	A	T	G	G	A	G	A	G	T	A	C
B-0812	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	T	C	A	G	A	G	G	A	G	A	G	G	A	T	G	G	A	G	A	G	T	A	C
B-0816	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	T	C	A	G	A	G	G	A	G	A	G	G	A	T	G	G	A	G	A	G	T	A	C
B-0815	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	T	C	A	G	A	G	G	T	G	A	G	C	A	T	G	G	A	G	A	G	T	A	C
B-4204	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	A	C	A	G	A	C	G	A	G	A	G	C	A	T	G	G	A	G	A	G	T	A	T
B-4201	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	A	C	A	G	A	G	G	A	G	A	G	C	A	T	G	G	A	G	A	G	T	A	C
B-4202	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	A	C	A	G	A	G	G	A	G	A	G	C	A	T	G	G	A	G	A	G	T	A	C
B-4205	A	G	G	A	A	C	G	C	C	A	C	G	T	G	C	A	T	A	T	C	T	A	C	A	G	A	G	G	A	G	A	G	C	A	T	G	G	A	G	A	G	T	A	C
B-0814	A	G	G	A	A	C	G	C	C	A	C	G	T	G	T	A	C	C	T	C	T	T	C	A	G	A	G	G	A	G	A	G	C	A	T	G	G	A	G	A	G	T	A	C
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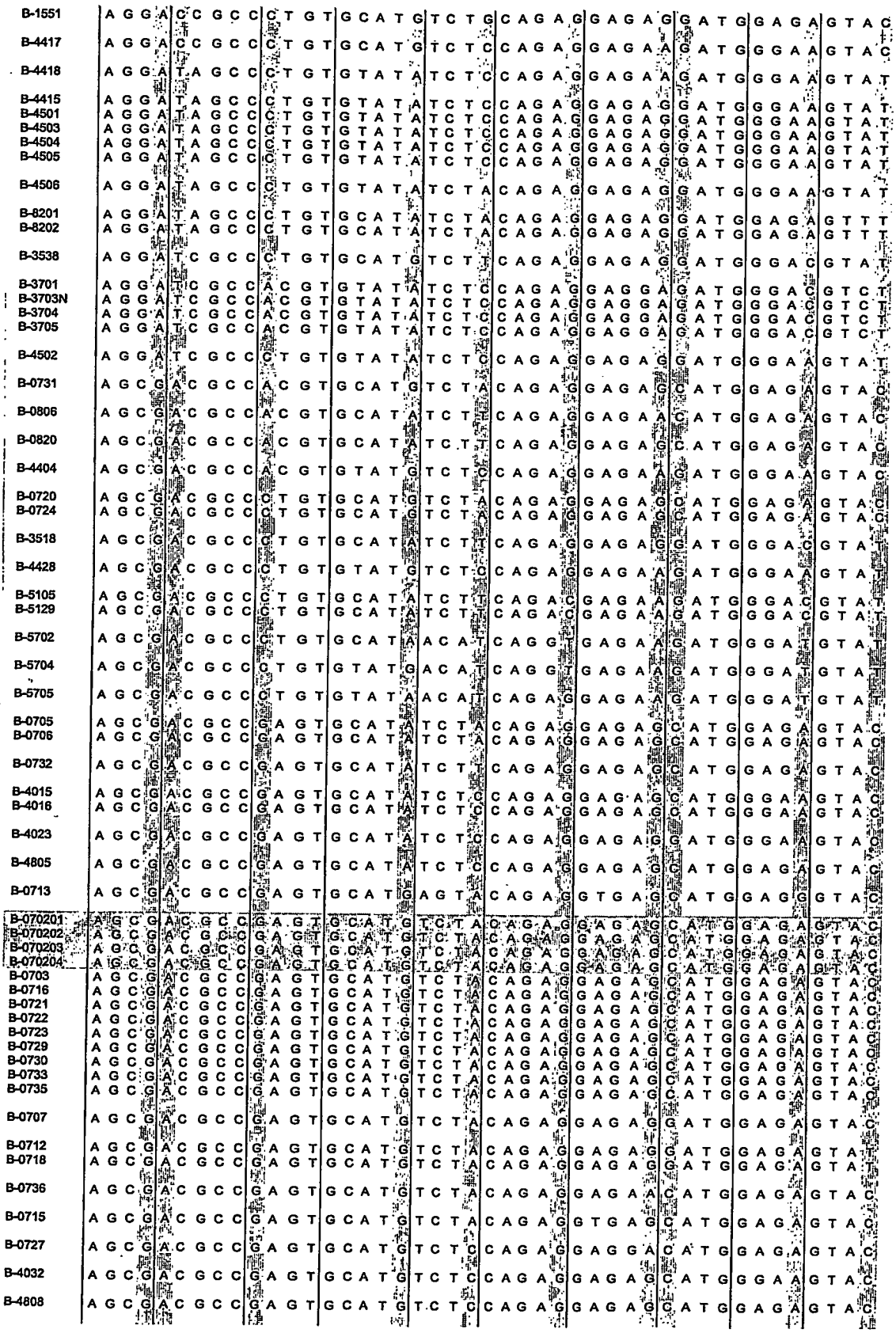
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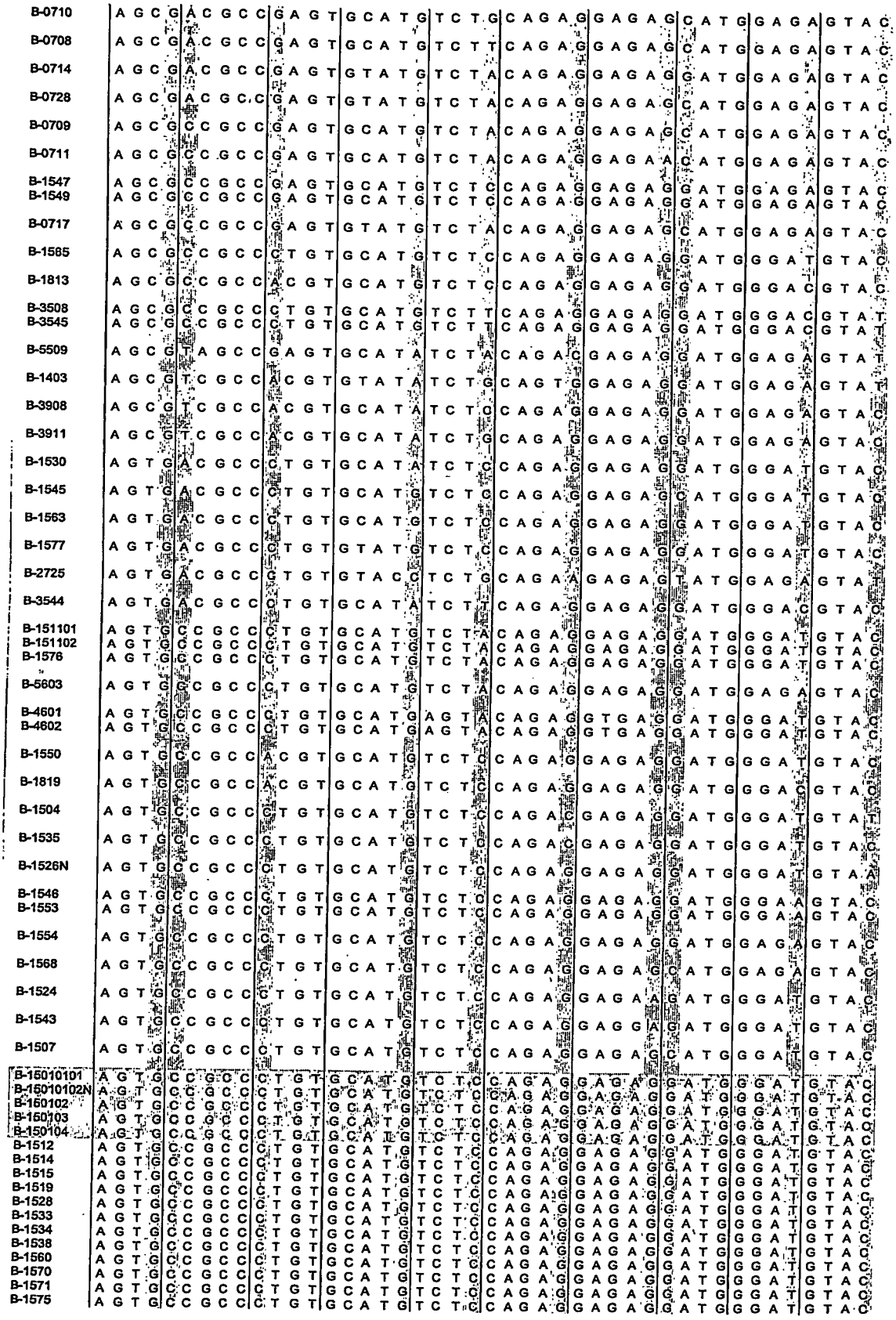
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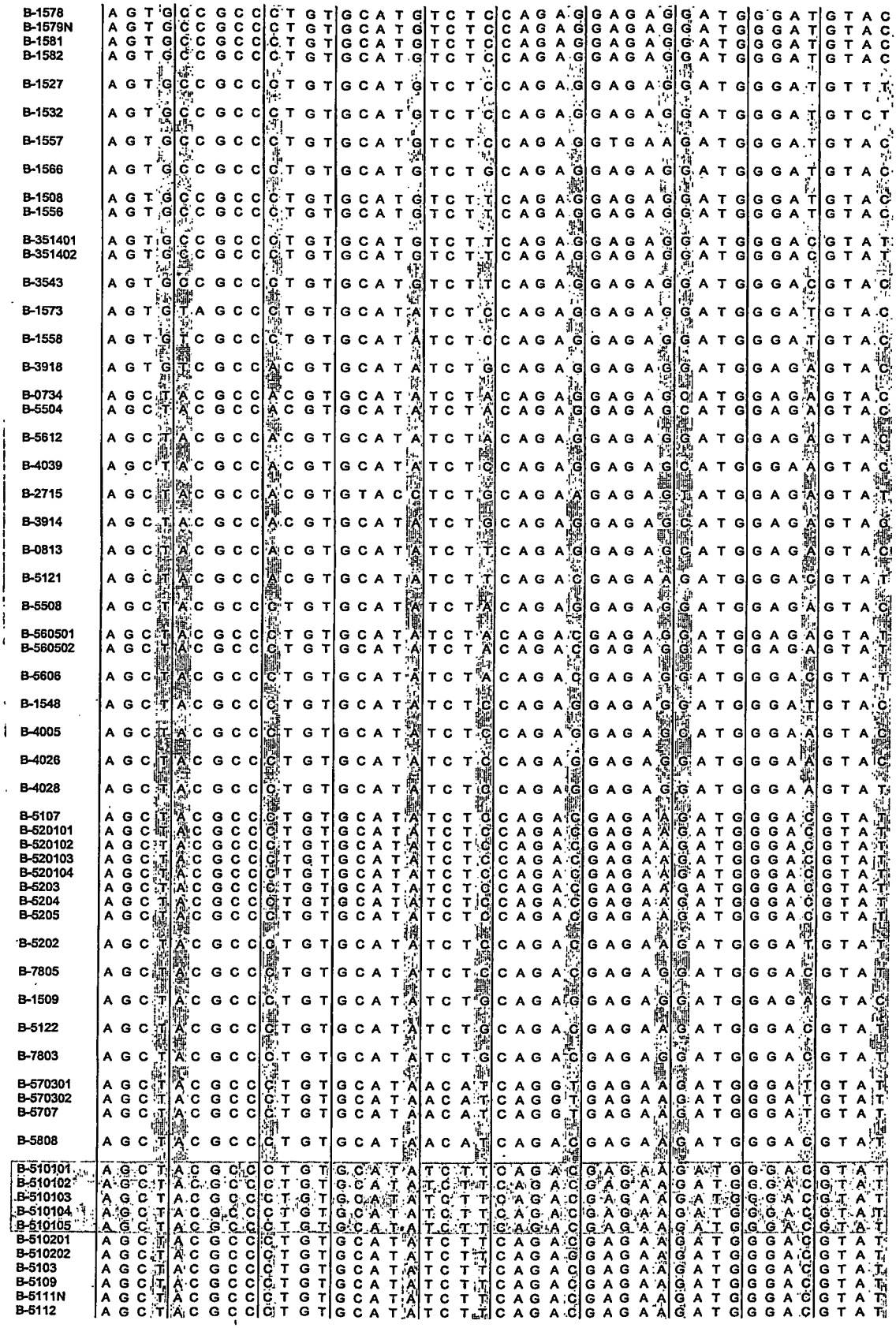
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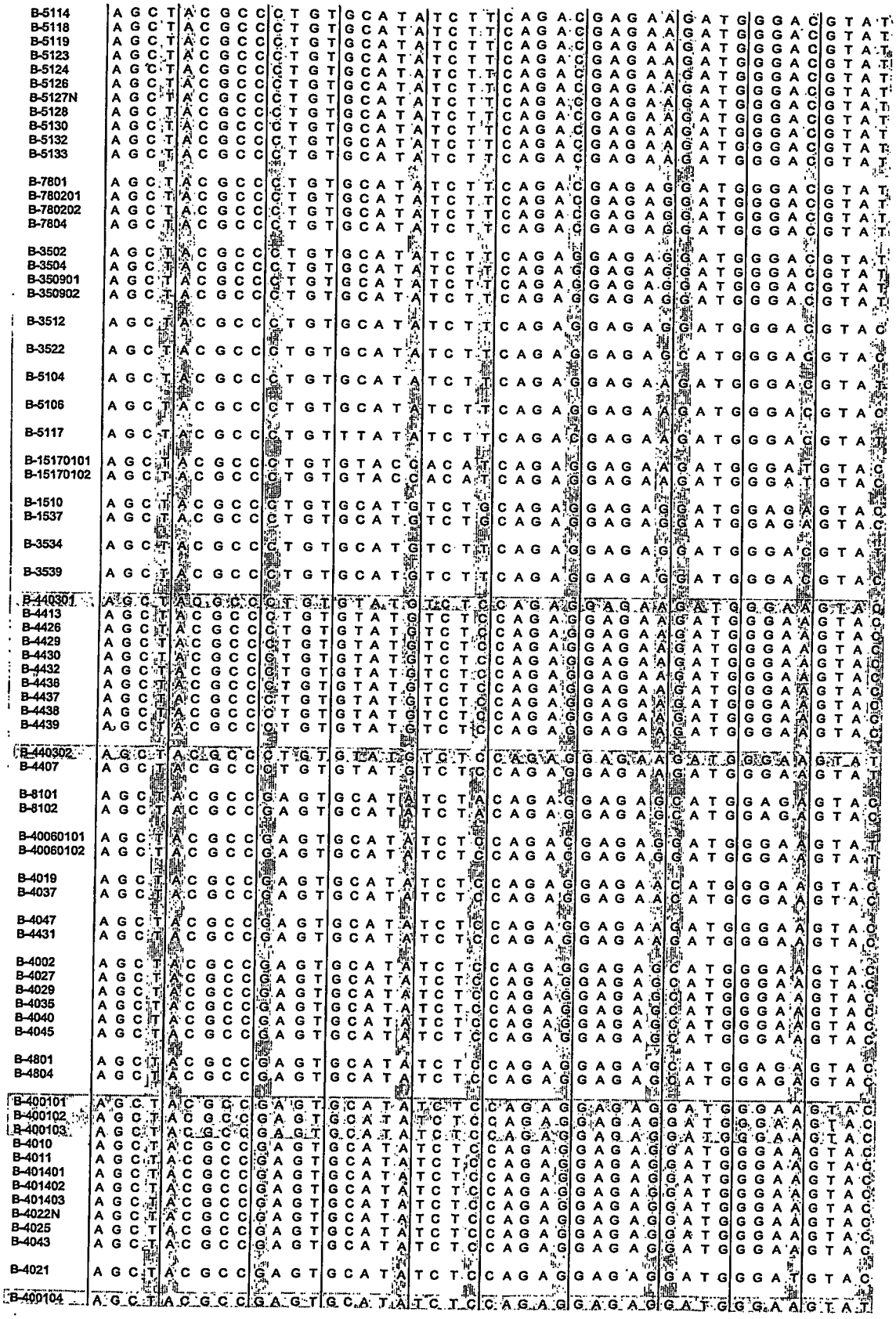

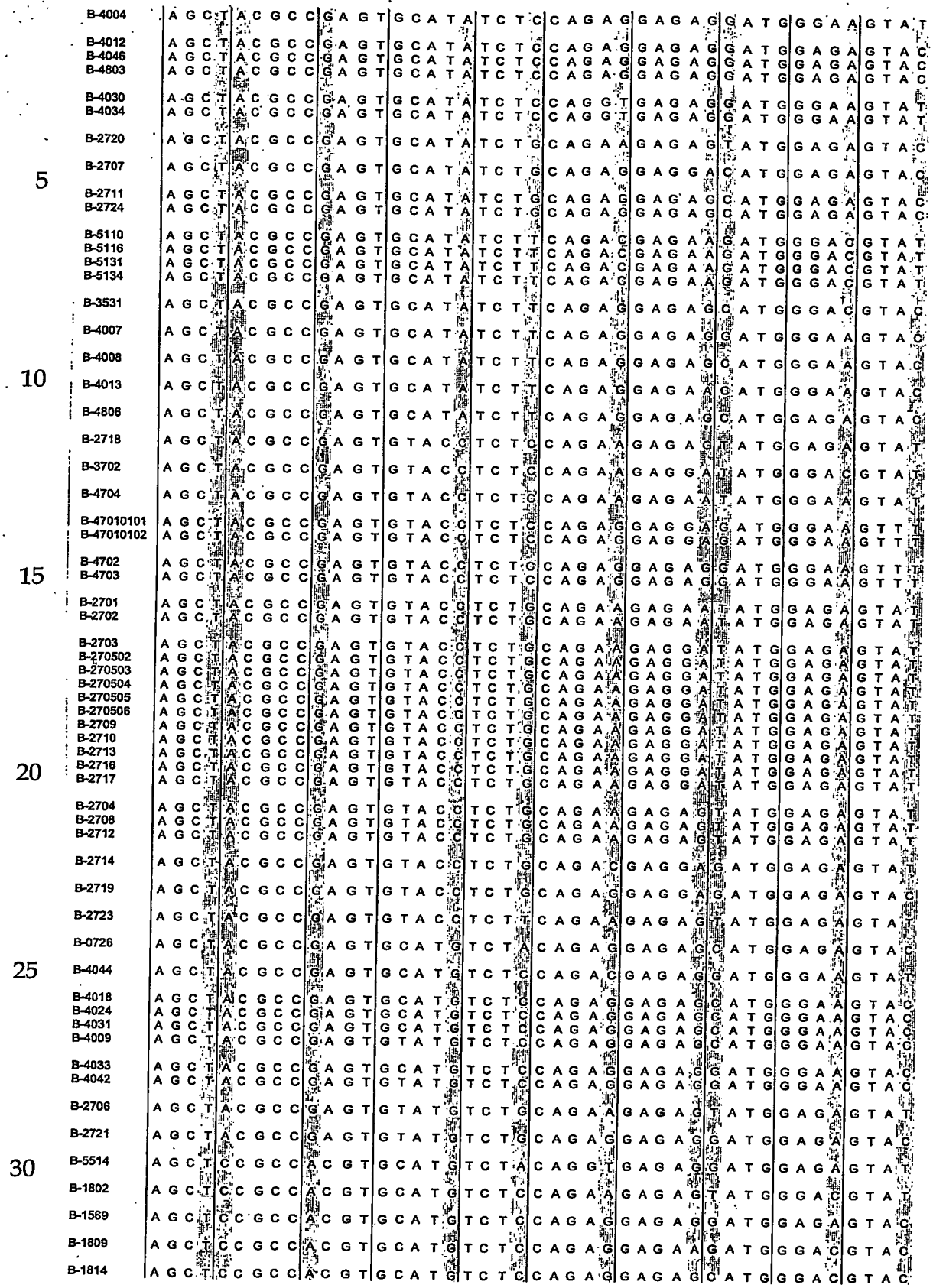
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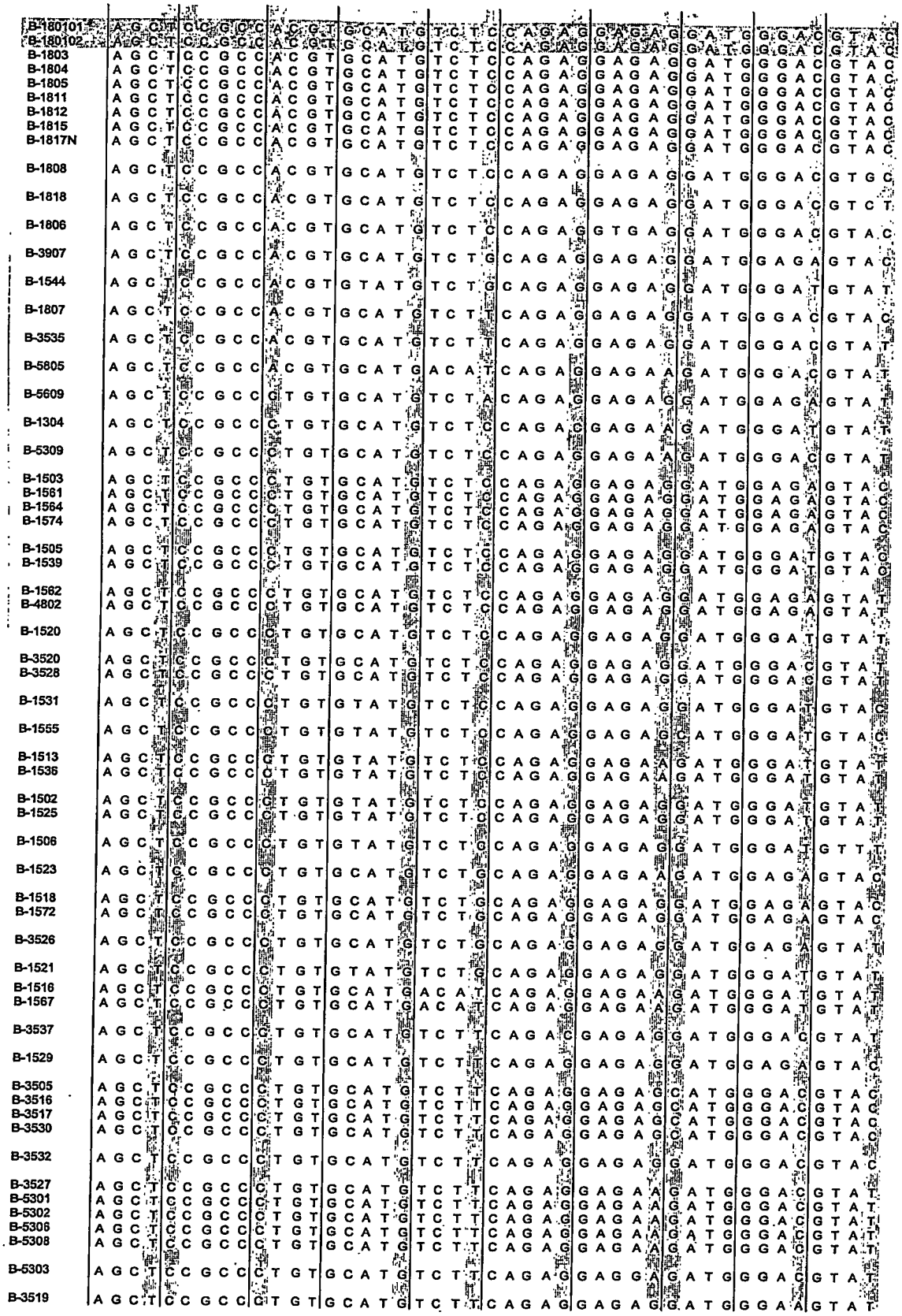

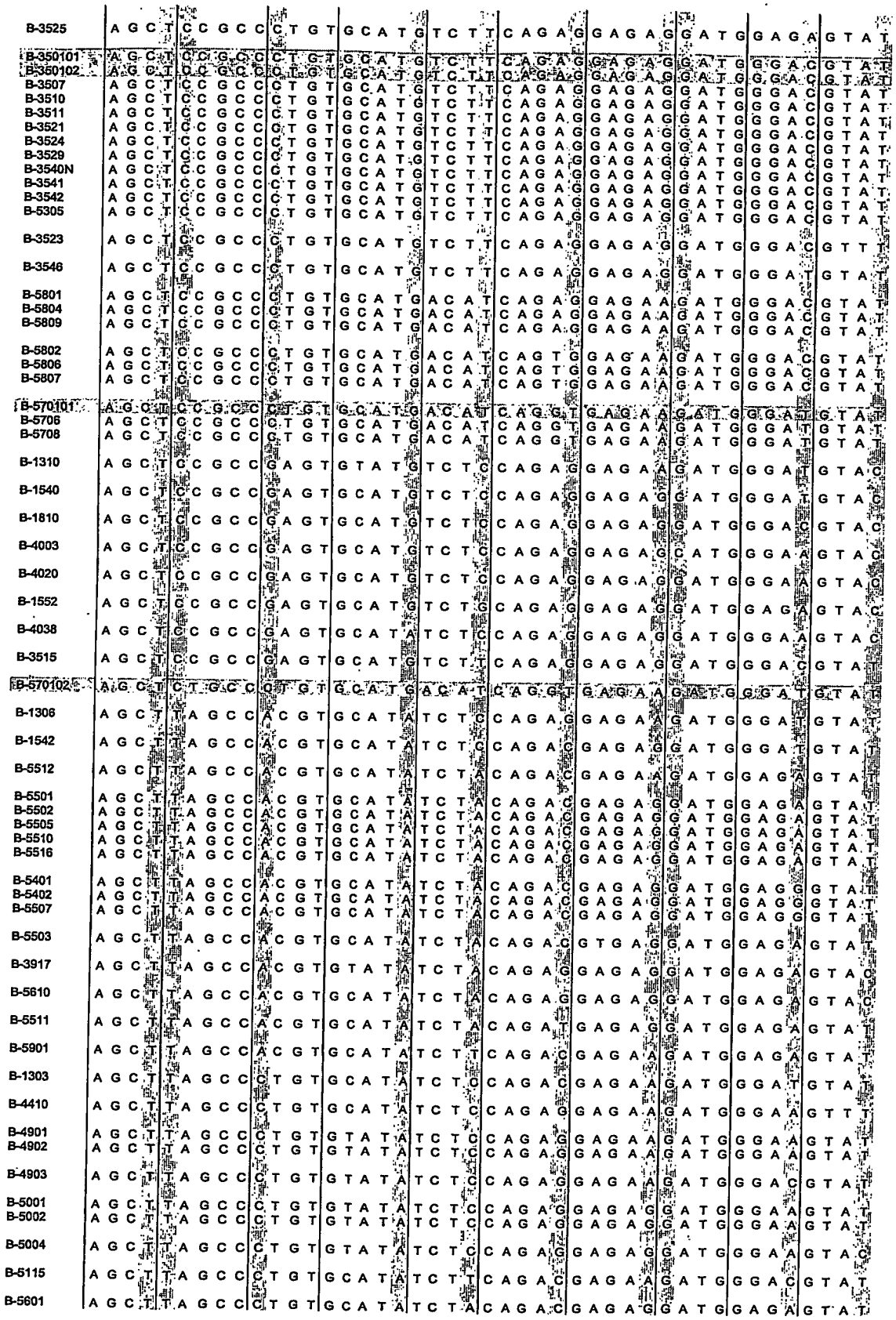
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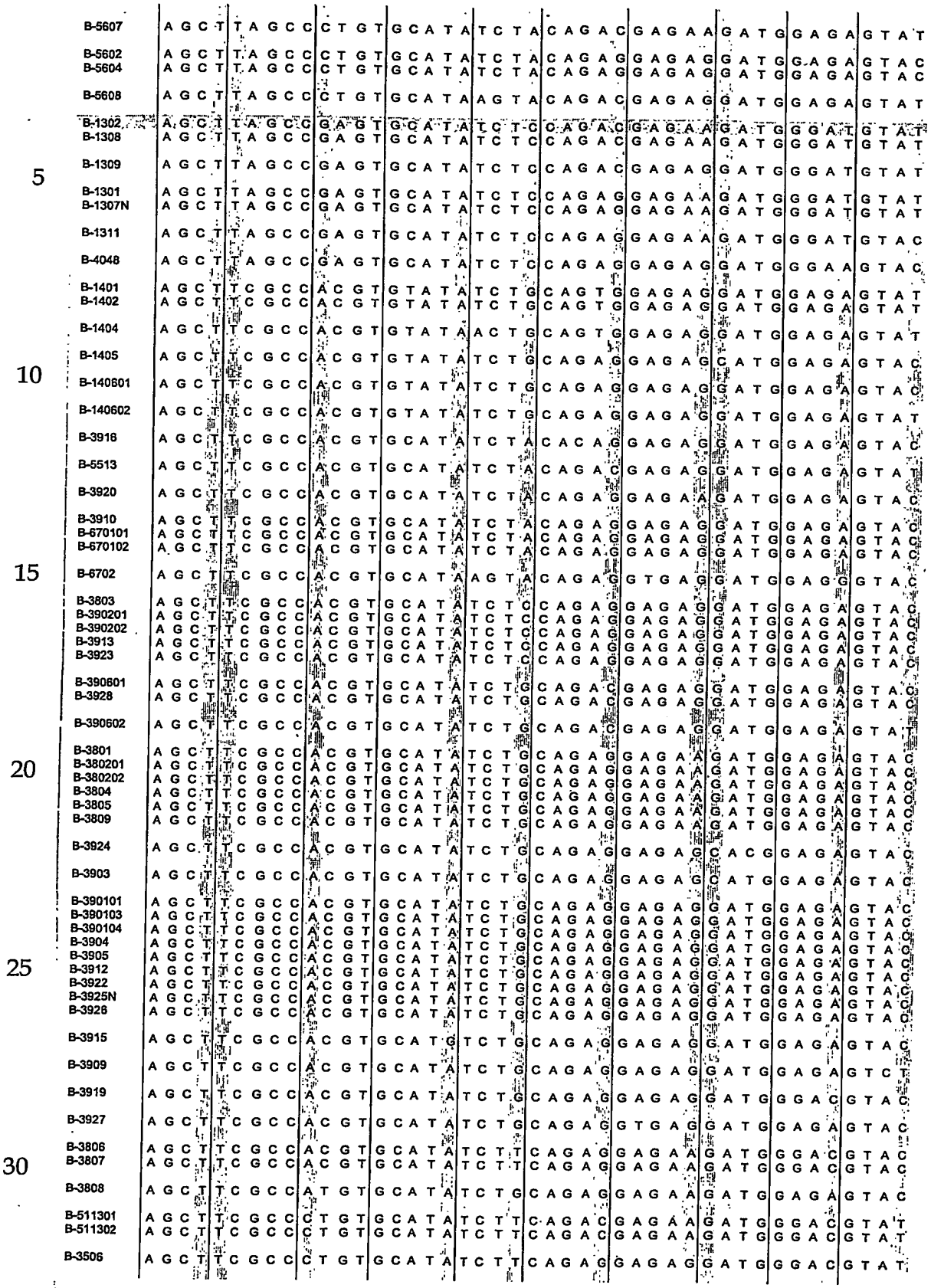


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TABLE XII

Position in cDNA	125	126	127	128	193	194	195	196	197	198	199	200	224	225	226	227	261	262	263	264	283	284	285	286	306	307	308	309	318	319	320	321	344	345	346	347	348	349	350	351	352	353	354	355
DRB1-070101	A	T	A	A	G	A	G	T	T	C	G	T	A	G	T	A	C	G	A	G	G	A	C	A	A	C	A	G	A	G	G	T	G	G	G	T	T	G	G	T				
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DRB1-0704	A	T	A	A	G	A	G	T	T	C	G	T	A	G	T	A	C	G	A	G	G	A	C	A	A	C	A	G	A	G	G	T	G	G	G	T	T	G	G	T				
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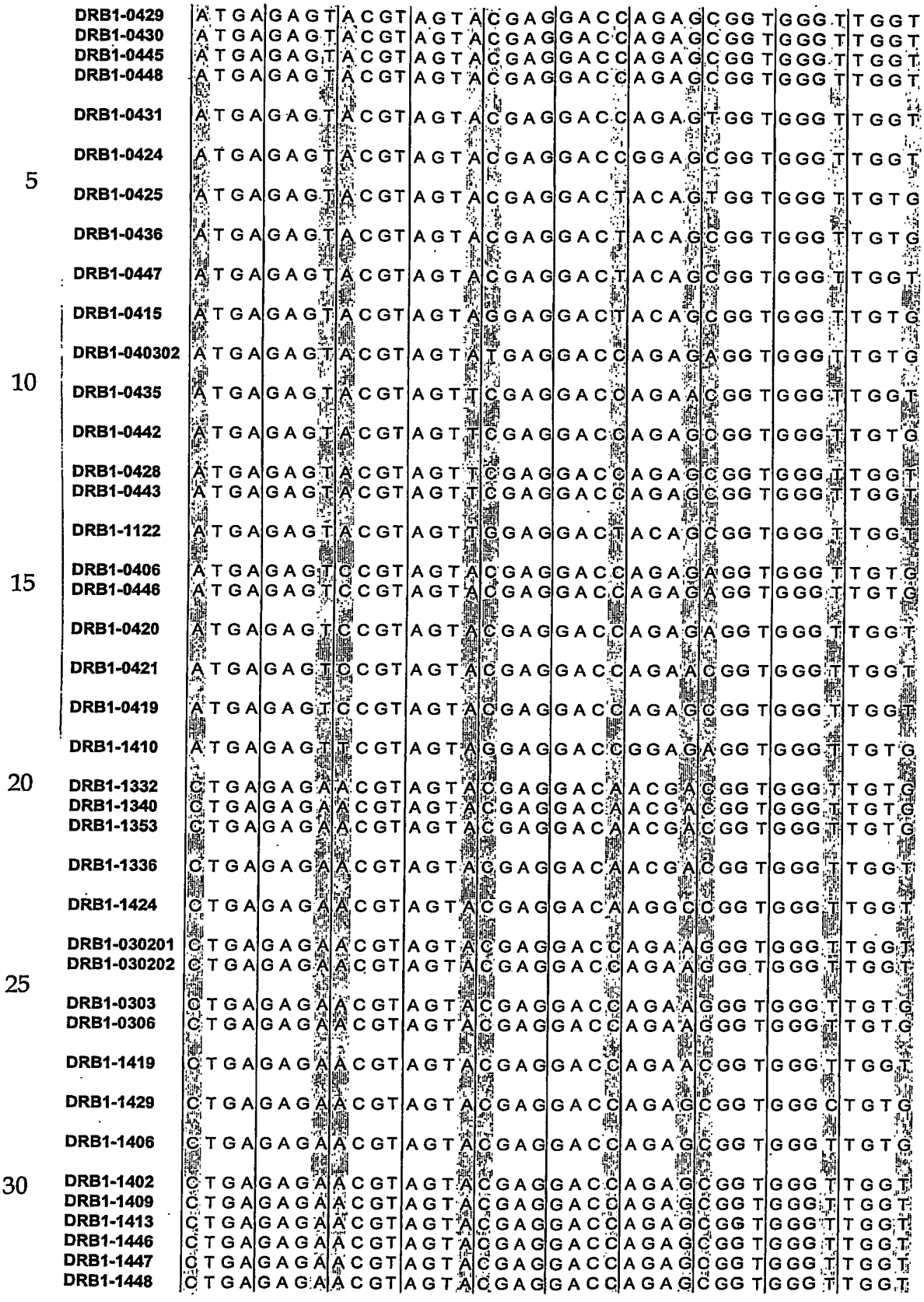
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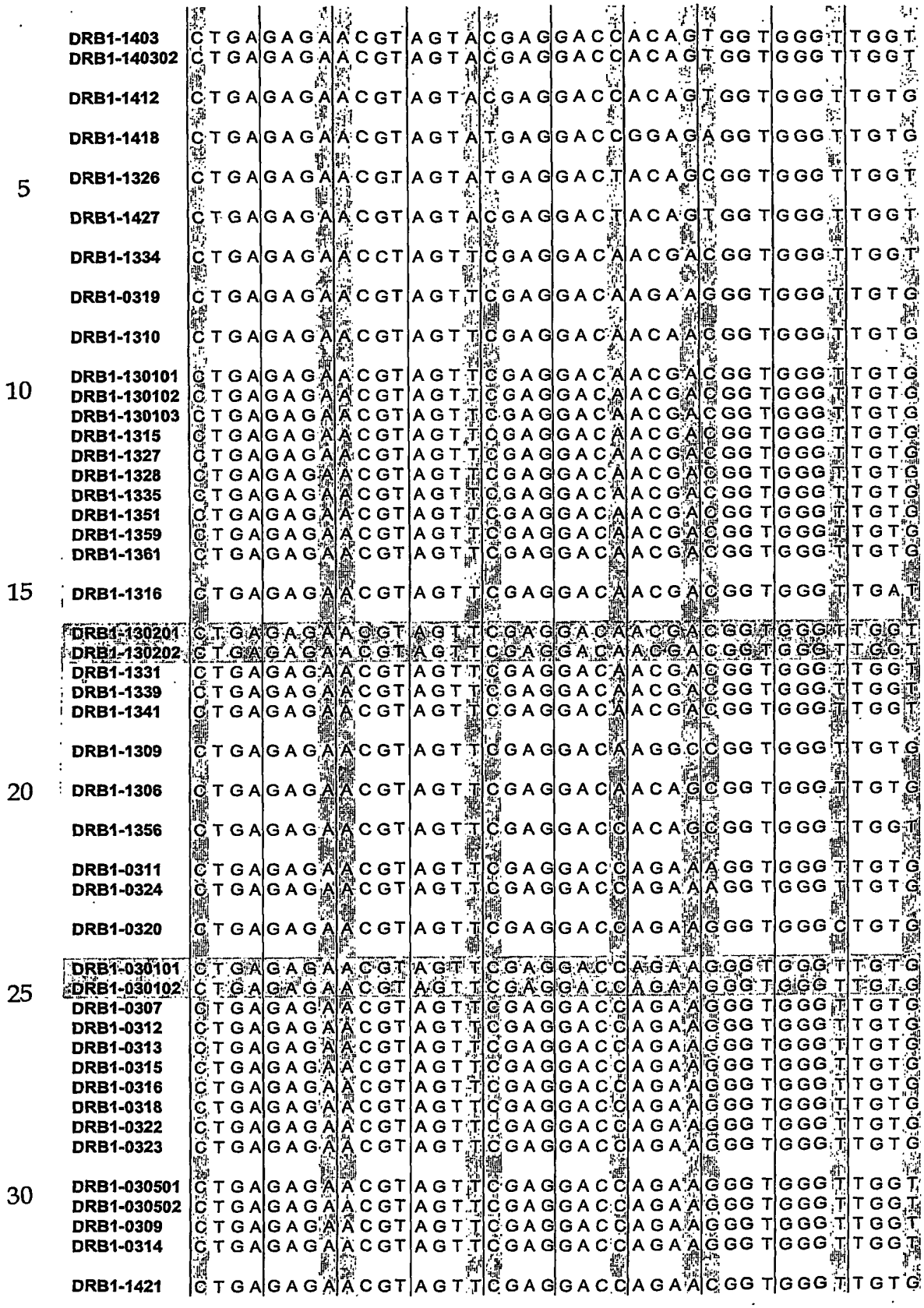
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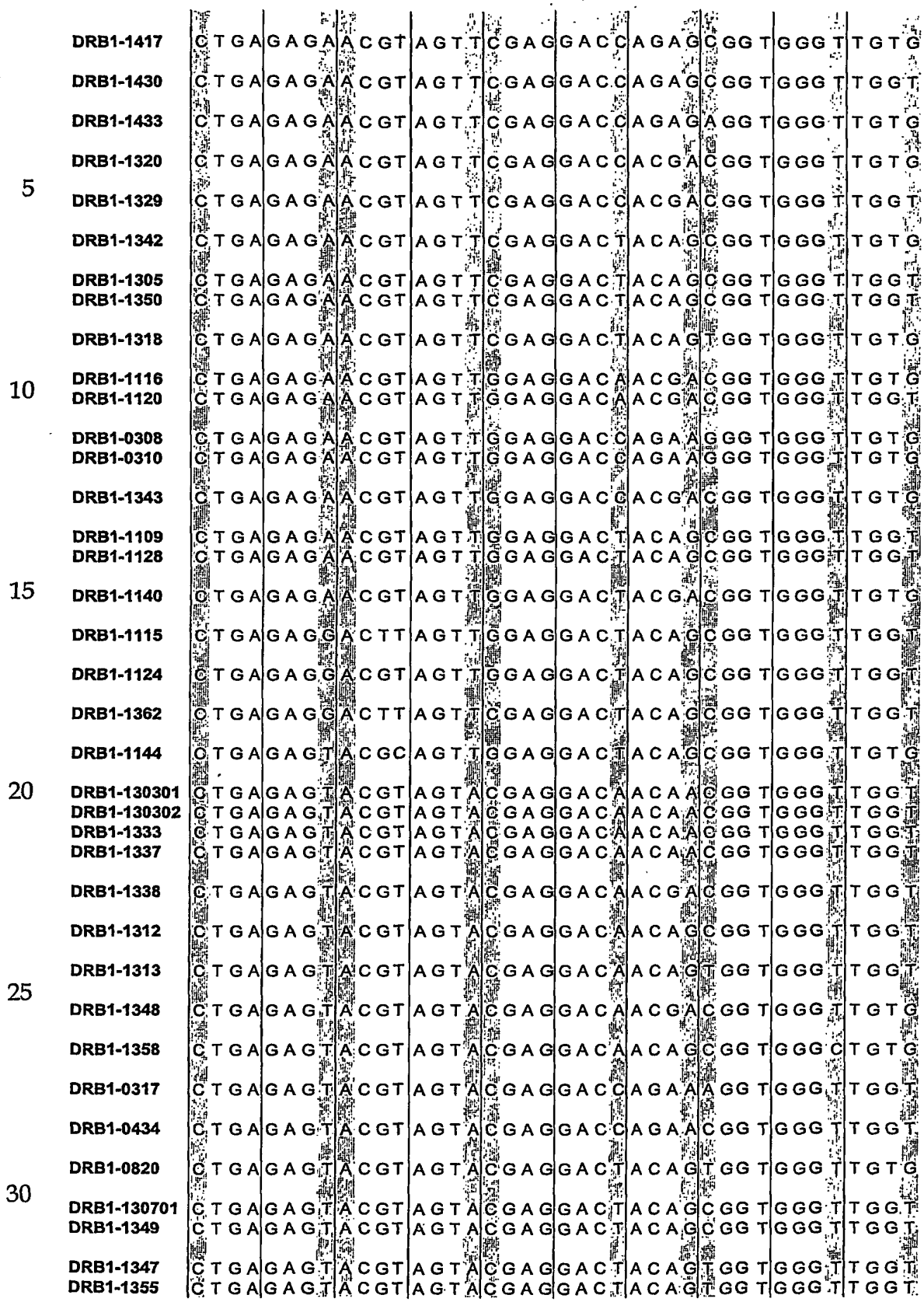
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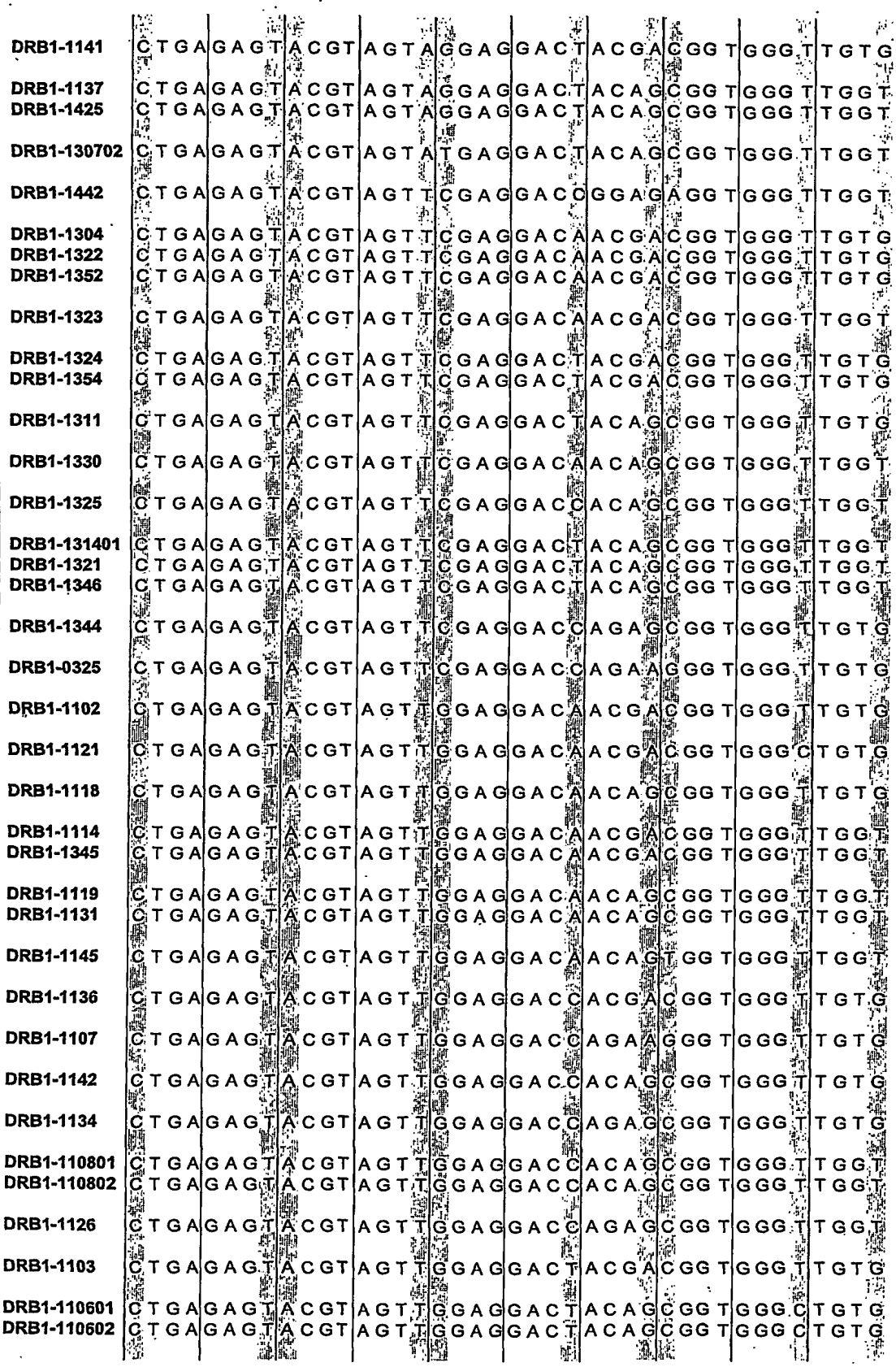
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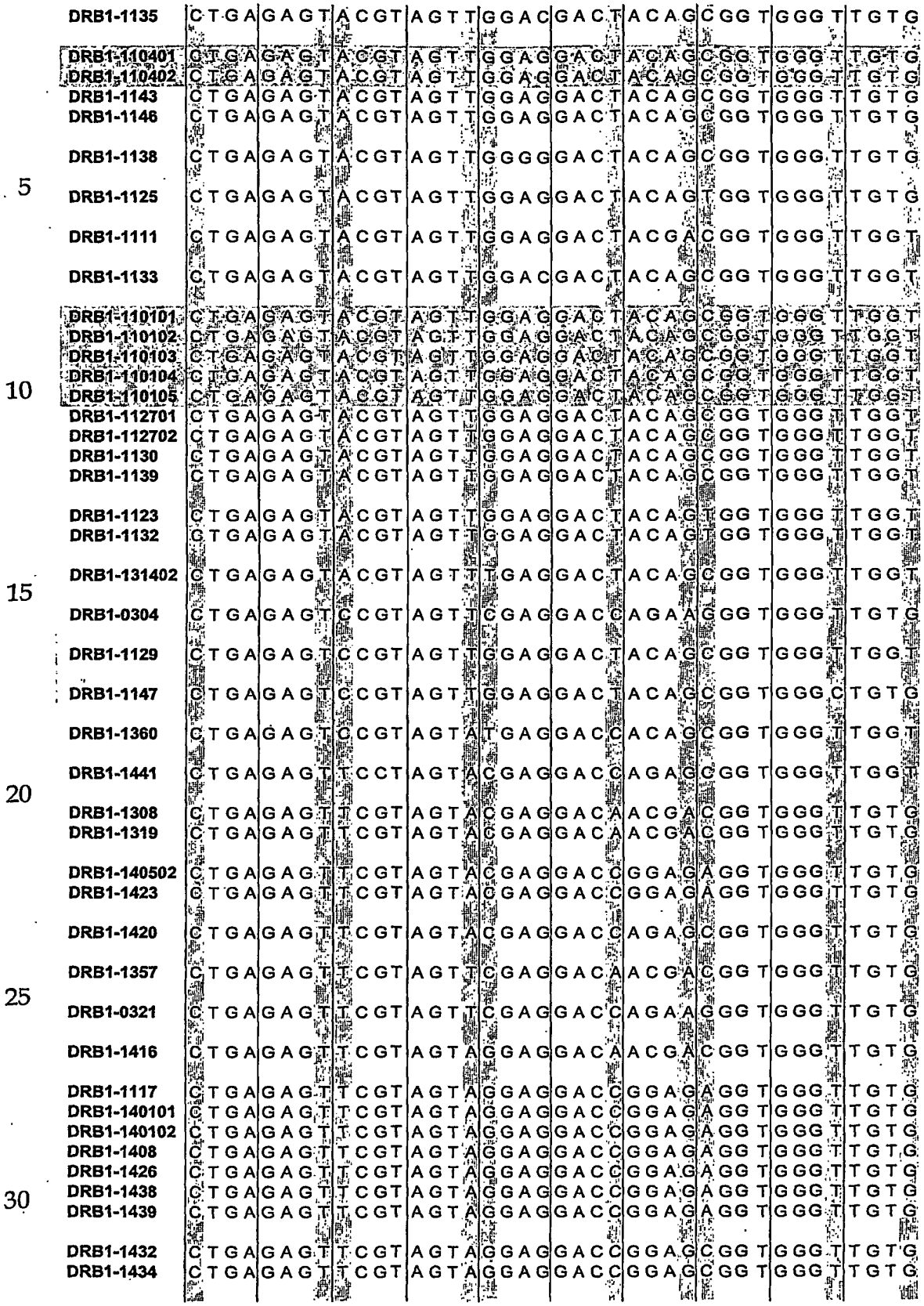
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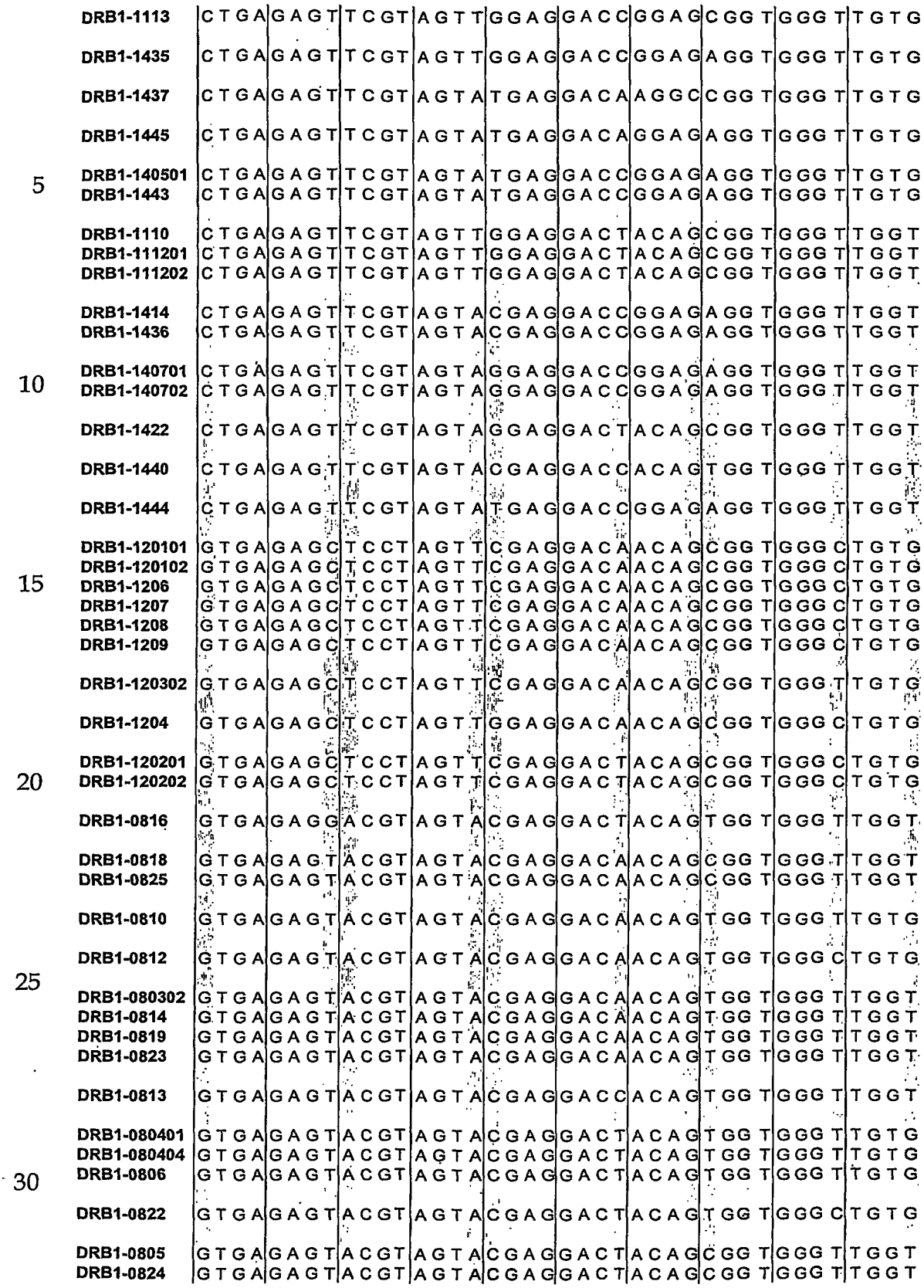
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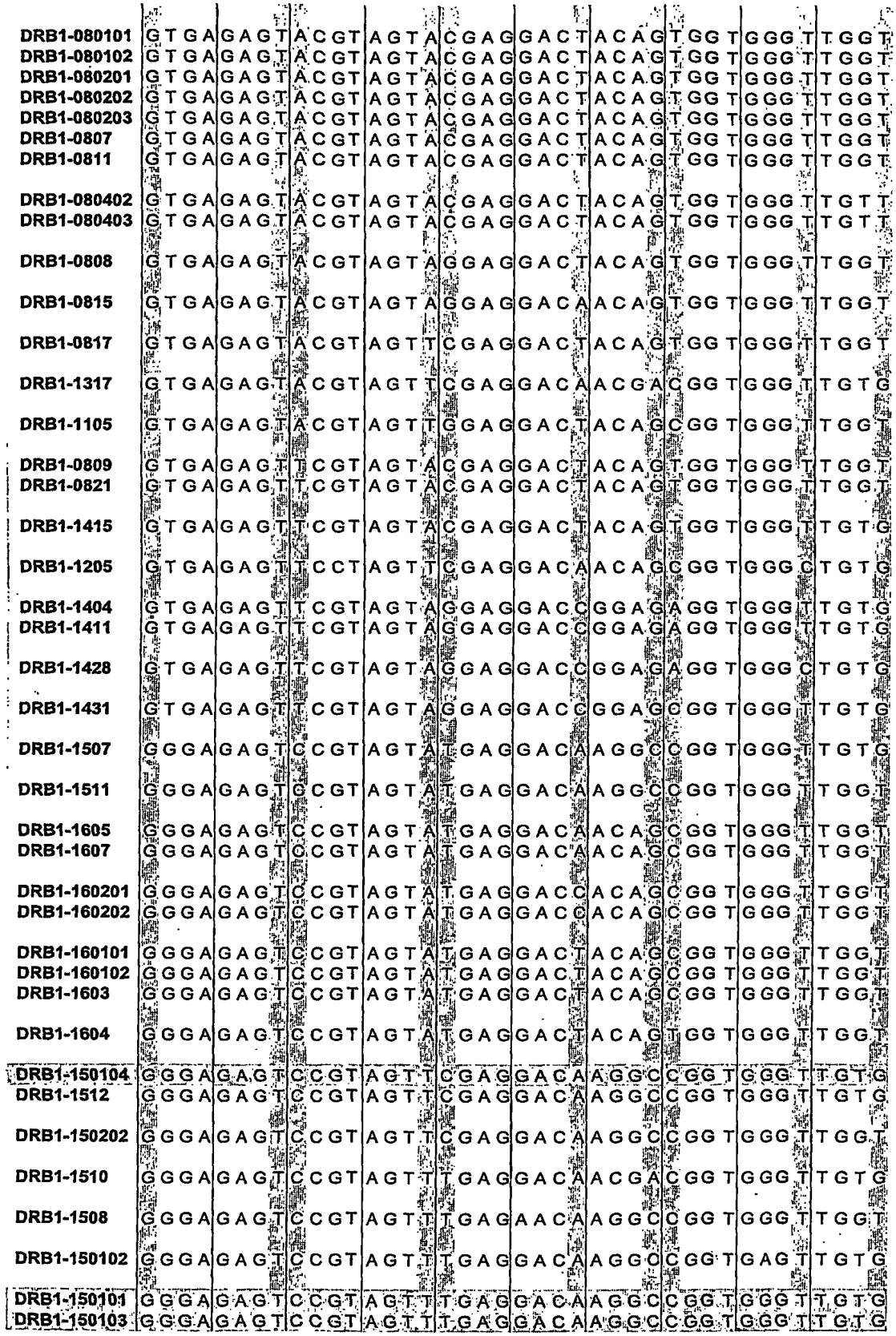
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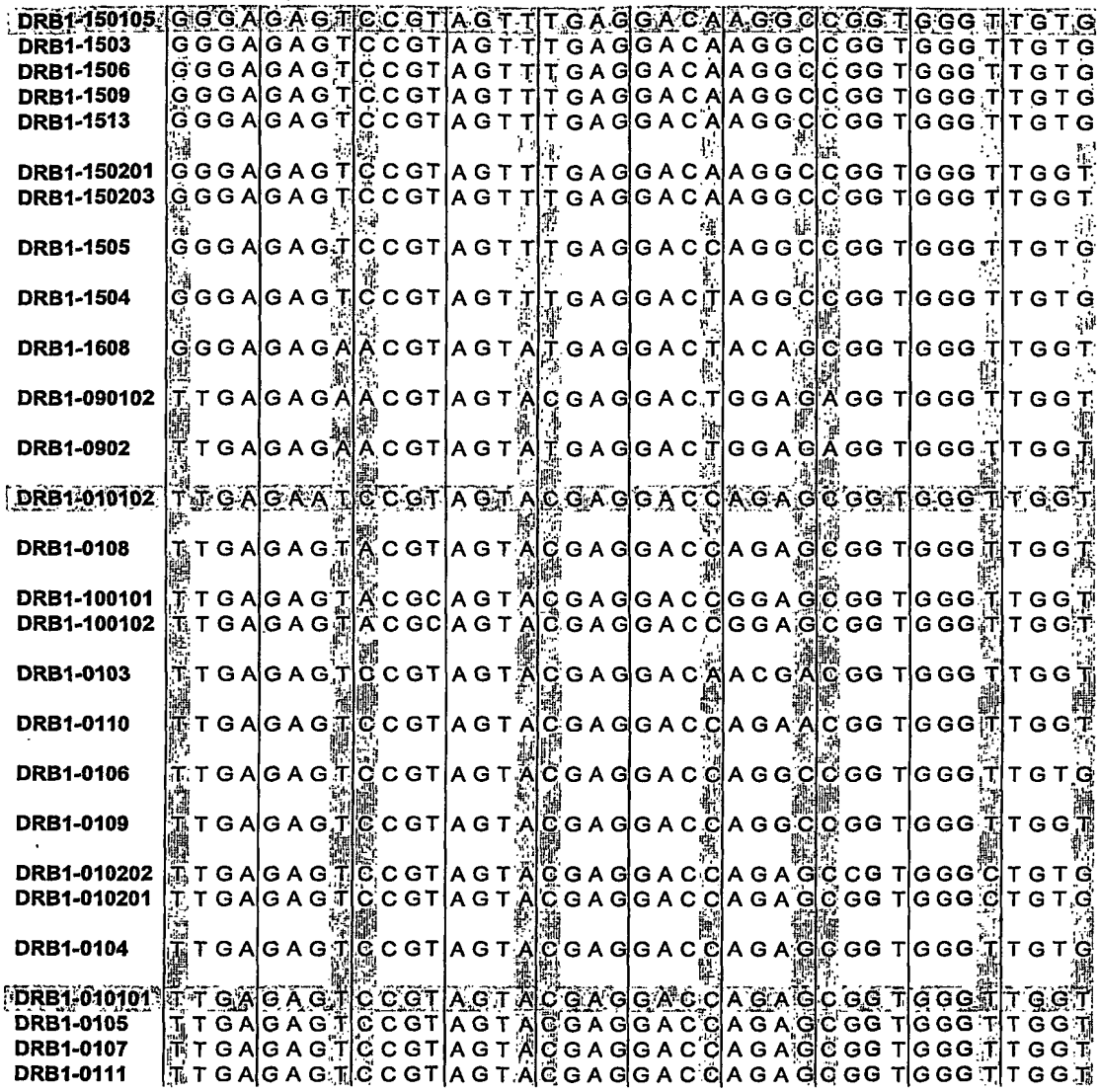
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DRB1-0111 TTGAGAGTCCGTAGTACGAGGACCAGAGCGGTGGGTTGGT

General strategy for medium resolution typing is described below:
For medium resolution typing a maximally informative set of marker positions were determined. These consist of positions 98, 414, 539, 282, 571, 368, 256, 292, 238, 270, 453, 527, 502, 81, 268, 559, 92, 123 and 396 of HLA-A (numbering starts at the transcription start position of exon 1), positions 539, 419, 559, 412, 272, 362, 302, 363, 206, 369, 259, 97, 583, 292, 222, 527, 418, 435 and 571 of HLA-B (numbering starts at the transcription start position of exon 1), and positions 125, 196, 197, 227, 261, 286, 299, 308, 341 and 345 of HLA-DRB1 (numbering starts at the transcription start position of exon 1).

In general, the order of the positions is from the most informative to the least informative with respect to the selection criteria of frequent and rare HLA alleles (see list of frequent HLA alleles above). Thus the ten markers (HLA-A and HLA-B) that were selected for the fine typing strategy constitute the first ten markers of the set of 19 markers for the single pass classification into frequent and rare HLA alleles (HLA-A and HLA-B). Like with sequence-based HLA typing there are heterozygous combinations of HLA alleles that can not be resolved. However, there are fewer ambiguities with this method due to the mini-haplotypes that are provided.

Another object of the present invention is the use of said methodology of the invention is for screening of tissue donors, for example, bone marrow donors in registries for frequent and rare HLA types.

The description of the HLA alleles is based on the Anthony Nolan database (www.ebi.ac.uk/imgt/hla/).

In addition to the aforementioned method, the invention includes yet other arrangements which will emerge from the description that follows, which refers to examples of supports according to the invention, as well as the annexed figures and tables, wherein:

Figure 1 describes 19 positions covered by mini-haplotyping assays for discrimination of HLA-A mapped onto the HLA-A allele A*010101 as reference. Black boxes indicate an extension position while grey boxes indicate polymorphisms that are captured by the annealing of the respective primer of the primer pool. Pools are used in forward and reverse. Numbering is according to the transcription start of the cDNA.

Figure 2 describes 19 positions covered by mini-haplotyping assays for discrimination of HLA-B mapped onto the HLA-B allele B*070201 as reference. Black boxes indicate an extension position while grey boxes indicate polymorphisms that are captured by the annealing of the respective primer of the primer pool. Pools are used in forward and reverse. Numbering is according to the transcription start of the cDNA.

Figure 3 describes 10 positions covered by mini-haplotyping assays for discrimination of HLA-DRB1 mapped onto the HLA-DRB1 allele DRB1*0101 as reference. Black boxes indicate an extension position while grey boxes indicate polymorphisms that are captured by the annealing of the respective primer of the primer pool. Pools are used in forward and reverse. Numbering is according to the transcription start of the cDNA.

Figure 4 describes 10 positions covered by mini-haplotyping assays for discrimination of HLA-A mapped onto the HLA-A allele A*010101 as reference for the distinction of subgroups that can then be further analysed. Black boxes indicate an extension position while grey boxes indicate polymorphisms that are captured by the annealing of the respective primer of the primer pool. Pools are used in forward and reverse. Numbering is according to the transcription start of the cDNA.

Figure 5 describes 10 positions covered by mini-haplotyping assays for discrimination of HLA-B mapped onto the HLA-B allele B*070201 as reference for the distinction of subgroups that can then be further analysed. Black boxes indicate

an extension position while grey boxes indicate polymorphisms that are captured by the annealing of the respective primer of the primer pool. Pools are used in forward and reverse. Numbering is according to the transcription start of the cDNA.

Figure 6 shows genotyping results of a CEPH family (1418, 01 = father, 02 = mother, 03 = child, 04 = child) for position HLA-B_272. 1407,3 Da corresponds to the addition of C to primer 6, 7, 8, or 9; 1422,3 Da corresponds to the addition of T to primer 6, 7, 8, or 9; 1431,4 Da/ 1430,9 Da corresponds to the addition of A to primer 6, 7, 8, or 9; and 1447,4 Da/ 1448,5 Da corresponds to the addition of G to primer 6, 7, 8, or 9.

Table I represents HLA-A alleles captured by the 10 markers in the different subgroups and additional positions that have to be typed to resolve the subgroups.

Table II represents HLA-B alleles captured by the 10 markers in the different subgroups and additional positions that have to be typed to resolve the subgroups.

Table III represents HLA-DRB1 alleles captured by the 10 markers in the different subgroups and additional positions that have to be typed to resolve the subgroups.

Table IV represents the list of the individual primers that are required to constitute the pools for mini-haplotyping of HLA-A (19 markers). The 10 markers required for the creation of subgroups are also contained. ^ refers to the base used to attach the mass/charge tag, CT refers to the mass difference of the mass/charge tag, sp means phosphorothioate group. The product analysed by mass spectrometry includes the base 5'of the most 5' phosphorothioate (sp).

Table V represents the list of the individual primers that are required to constitute the pools for mini-haplotyping of HLA-B (19 markers). The 10 markers required for the creation of subgroups are also contained. ^ refers to the base used to attach the mass/charge tag, CT refers to the mass difference of the mass/charge tag, sp

means phosphorothioate group. The product analysed by mass spectrometry includes the base 5'of the most 5' sp.

Table VI represents the list of the individual primers that are required to constitute the pools for mini-haplotyping of HLA-DRB1 (10 markers). ^ refers to the base used to attach the mass/charge tag, CT refers to the mass difference of the mass/charge tag, sp means phosphorothioate group. The product analysed by mass spectrometry includes the base 5'of the most 5' sp.

Table VII represents the resolution that can be generated with the 19 markers for the distinction of the frequent HLA alleles in HLA-A.

Table VIII represents the resolution that can be generated with the 19 markers for the distinction of the frequent HLA alleles in HLA-B.

Table IX represents the resolution that can be generated with the 10 markers for the distinction of the frequent HLA alleles in HLA-DRB1.

Table X represents the list of HLA-A alleles that are resolved with the 10 markers for the creation of subgroups. Each subgroup is separated by an empty line. Frequent alleles are shaded in darker grey, while lighter grey indicates the position that primers are extended onto.

Table XI represents the list of HLA-B alleles that are resolved with the 10 markers for the creation of subgroups. Each subgroup is separated by an empty line. Frequent alleles are shaded in darker grey, while lighter grey indicates the position that primers are extended onto.

Table XII represents the list of HLA-DRB1 alleles that are resolved with the 10 markers for the creation of subgroups. Each subgroup is separated by an empty line. Frequent alleles are shaded in darker grey, while lighter grey indicates the position that primers are extended onto.

Examples

Example 1: Mini-haplotyping at position 272 of HLA-B by the modified GOOD-Assay

A locus specific PCR product of exon 2 and exon 3 of HLA-B is amplified with a set of primers published by the International Histocompatibility Working Group, Technical Manuals (Hurly, Fernandes-Vina, Gao, Middleton, Noreen, Ren and Smith; www.ihwg.org/tmanual/Tmcontents.htm). The PCR product is incubated with SAP to remove all excess dNTPs. Then a single base primer extension at position 272 in the PCR amplicon is carried out. The set of primers, to generate the mini-haplotypes is shown in Table V. Thereafter a 5'phosphodiesterase digest is applied to reduce the primers to a core sequence. After alkylation of the DNA backbone of the mini-haplotype fragments the products are transferred onto a MALDI target pre-coated with matrix. Alternatively the matrix solution can be mixed with the samples and transferred onto the MALDI target to dry. The MALDI target is introduced into a MALDI mass spectrometer and analysed. The mass spectra show one or two mass peaks and that correspond to specific mini-haplotypes.

PCR:

Forward primer, BAmp1 5′-G GGT CCC AGT TCT AAA GTC CCC ACG-3′(1.875 pmol), reverse primer, BAmp2 5′-CC ATC CCC GGC GAC CTA TAG GAG ATG-3′ (1.875 pmol) an BAmp3 5'-AGG CCA TCC CGG CGG GCG ATC TAT-3' (1.875 pmol), 0.25 μl 10x PCR buffer (HiFi Platinum Taq)), 0.3 μl $MgSO_4$ (50 mM), 0.2 μl of a mix of each dCTP, dATP, dGTP and dTTP (2 mM each), 0.25U engineered DNA polymerase (HiFi Platinum DNA Polymerase; Invitrogen) and 5 ng DNA fill to 3 μl with water. Cycling: 1. 94°C 3 min, 2. 94°C 20 sec, 3. 64°C 30 sec, 4. 72°C 30 sec, steps 2 to 4 are repeated 35 times, 5. 72°C 5 min.

SAP digest:

1.75 µl of 50 mM Tris-HCl and 0.25 µl SAP (USB corporation, Cleveland, USA) are to add to the PCR product and this has to be incubated for 60 min at 37°C, followed by an incubation at 90°C for 10 min to denature the SAP enzyme.

Single Base Primer Extension:

To the SAP treated PCR product 2 µl of an extension mix is to add. This mix contains 15 mM $MgCl_2$, 0.1 mM of each of the four α–S-ddNTPs, 5 pmol of the extension primers set and 0,4 U of Thermosequenase. Cycling: 1. 94°C 2 min, 2. 94°C 15 sec, 3. 58°C 20 sec, 4. 72°C 20 sec, steps 2 to 4 are repeated 50 times.

PDE digest:

To the extension product has to be added 0.5 ul 0.5 M acetic acid and 1.5 µl PDE (5.1U) and incubate for at lease 120 min at 37 °C.

Alkylation:

The alkylation is carried out by adding 21 µl of an alkylation mix and incubate for 15 min at 40°C. Th alkylation mix contains 377 parts water free acetonitrile, 15 parts of 2M triethylamine/CO_2 (pH ~7.5), 75 parts 2mM Tris-HCl and 174 parts of methyliodine.

The alkylation is to stopped by adding 10 µl deionised water. 5 µl of the resulting upper phase are to dilute in 10 µl 40% acetonitrile.

For MALDI target preparation and measurement with the MALDI mass spectrometer 0.5 µl of the final dilution are transferred onto a MALDI target pre-coated with matrix (α-cyano-4-hydroxycinnamic acid methyl ester). Measurement was carried out in a Bruker Autoflex with typically -18 kV acceleration voltage, pulsed ion extraction with a delay of 200 ns, and detection in linear detection mode. Results for CEPH family 1418 are shown in figure 6.

Example 2: HLA-DR typing by the GOOD-Assay

A locus specific PCR for HLA-DRB is carried out. Therefore a set of allele-specific primers as listed below is used. These primers are published by J. Wu et al. in http://www.ihwg.org/tmanual/TMcontents.htm Chapter 10-B.

Name	Sequence
Amp1_DRB1_f20	5'-TTCTTGTGGSAGCTTAAGTT-3'
Amp2_DRB1_f21	5'-TTCCTGTGGCAGCCTAAGAGG-3'
Amp3_DRB1_f22	5'-CACGTTTCTTGGAGTACTCTAB-3'
Amp3-2_DRB1_f23	5'-CGTTTCTTGGAGTACTCTACGGG-3'
Amp3-3_DRB1_f23	5'-CGTTTCTTGGAGTACTCTACGTC-3'
Amp4_DRB1_f21	5'-GTTTCTTGGAGCAGGTTAAAC-3'
DR7_DRB1_f20	5'-CCTGTGGCAGGGTAARTATA-3'
DR9_DRB1_f18	5'-CCCAACCACGTTTCTTGA-3'
DR10_DRB1_f19	5'-AGACCACGTTTCTTGGAGG-3'
AmpB_DRB1_r18	5'-TCGCCGCTGCACYGTGAA-3'

This set of primers carries a high risk of co-amplifying genes for the other HLA-DRB chains, which results in unclear results. However, this is currently the best available option for the PCR of HLA-DRB1. In order to resolve the problem an additional mini-haplotyping test can be added. The mini-haplotyping assay HLA-DRB_122-126 gives good resolution of HLA-DRB genes and allows the verification of results produced for typing of HLA-DRB1 PCR products. The identification of HLA-DRB1 genes is possible, as well as the identification of other amplified HLA-DRB genes which are present is possible. The set of primers listed below is used for the primer extension reaction. The details of the protocol are identical to example 1.

Name	Sequence	CT	Masses Primer	A	C	G	T
HLADR_1221_2f20	TGAAGAAATGACACTCAspTspG*spT	0	1487,5	-	-	-	1805,7
HLADR_1222_2f20	TGCAGAAATAGCACTCGspTspG*spT	0	1503,5	-	-	-	1821,7
HLADR_1223_2f20	TGAAGAAATGACACTCAspGspG*spT	0	1512,5	-	-	-	1830,7
HLADR_1224_2f20	TGAAGAAATGACACTTAspTspA*spT	0	1471,5	-	-	-	1789,7
HLADR_1225_2f20	TGAAGAAATGACACTCCspCspT*spC	-14	1510,6	-	-	-	1814,8
HLADR_1226_2f20	TGAAGAAATRACACTCAspCspC*spC	-28	1418,4	1717,7	1693,6	1733,7	-
HLADR_1227_2f20	TGAAGAAATGACACTCAspTspA*spC	-14	1456,5	-	-	-	1760,7
HLADR_1228_2f20	TGAAGAAWTGACACTCAspGspA*spC	0	1481,5	-	-	-	1799,7
HLADR_1229_2f20	TGAGGAAATGACACTCAspCspA*spC	-14	1441,5	-	-	1770,8	1745,7
HLADR_12210_2f20	TGAAGATATGACACTCAspCspA*spC	-14	1441,5	-	-	1770,8	1745,7
HLADR_12211_2f20	TGAAGAAATGACAYTCAspAspA*spC	0	1465,5	-	-	-	1783,7

Of the thirteen possible mini-haplotypes, four represent genes other than HLA-DRB1. The mini-haplotype GTGTT (1821.7 Da), AACAC in sense direction, represents with 100% certainty co-amplification of the HLA-DRB9 gene. The mini-haplotype ATACT (1760.8 Da), AGTAT in sense direction, represent either all HLA-DRB1*07 alleles (except HLA-DRB1*070102) or co-amplification of the HLA-DRB5 gene. The type TGTGT (1745.7 Da), AGTGT in sense direction, correspond to co-amplification or all variations of the HLA-DRB4 or HLA-DRB6 genes. Finally the type AGACT (1799.7 Da), AGTCT in sense direction, represent besides HLA-DRB1*1130 and HLA-DRB1*1446 also co-amplification of all variants of HLA-DRB3 and HLA-DRB7 genes.